Appendix C

LITERATURE REVIEWS

RAW GROUND (03B)

The Raw Ground HACCP category includes ground product (e.g., ground beef, ground chicken), marinated products, injected products, and otherwise comminuted products.

Pathogens of Concern

From a public health perspective, the bacterial pathogens of most concern for raw ground products are *E. coli* O157:H7, *Salmonella* spp., *Campylobacter jejuni, Listeria monocytogenes, Clostridium perfringens* MMWR 43(8):1994, *Clostridium botulinum and Yersinia enterocolitica* (Zhao et al, 2001; Kennedy M, Angulo FJ, 1999; Troutt HF, Osburn BI, 1997).

E. coli O157:H7 is a major public health concern for ground products made from beef (FSIS, 2004) causing such severe diseases such as hemolytic anemia (Tapper et al, 1995) and thrombocytopenic purpura (Nguyen, 2007). It is well recognized that beef is a common source for this pathogen. Moreover, in recent years, several *E. coli* O157:H7 outbreaks have been linked to the consumption of undercooked ground beef patties (Clavero, 1998).

Salmonella is one of the leading causes of bacterial foodborne disease outbreaks in the United States (DuPont, 2007). Furthermore, most of the reported outbreaks are attributed to consumption of inadequately cooked, contaminated animal products (Goodfellow and Brown, 1978; Bean and Griffin, 1990; and Tauxe, 1991).

Campylobacter is the most common cause of acute bacterial gastroenteritis in humans worldwide (Mead et al., 1999). *Campylobacter* is a common contaminant of broiler carcasses in poultry processing plants (Atabay and Corry, 1997; Berrang and Dickens, 2000; Gonzalez et al., 2000). Studies have demonstrated high levels of *Campylobacter* on broiler chickens from the farm (Stern et al., 1995) and from retail chickens (Zhao et al., 2001). Consequently, undercooked and raw poultry meats are common vehicles for the transmission of human campylobacteriosis.

Listeria monocytogenes has been associated with numerous foodborne outbreaks worldwide. This bacterial pathogen accounts for 28% of the estimated foodborne deaths annually in the United States (Mead, 1999). For example, in 2002, there was a *Listeria monocytogenes* foodborne outbreak originating from fresh and frozen RTE chicken and turkey products that caused illness in more than 46 people, with seven deaths and three miscarriages (CDC, 2002). This bacterial pathogen is a significant public health concern for susceptible population groups such as pregnant women, the elderly, neonates, and immunocompromised individuals.

Clostridium perfringens and *Clostridium botulinum* are a concern for raw ground meat and poultry products because of their ability to form spores. *Clostridium perfringens* foodborne illness annually ranks among the most common foodborne disease in Europe and the United States. CDC reported, for 1973 through 1987, that meat and poultry continued their traditional roles as the most common food vehicles for *Clostridium perfringens* type A food poisoning in the United States. Beef accounted for about 30% of all *C. perfringens* foodborne outbreaks, while turkey and chicken together accounted for another 15% of the outbreaks (Doyle et al., 1997).

Yersiniosis is an infectious disease caused by a bacterium of the genus *Yersinia.* In the United States, most human illness is caused by one species, *Y. enterocolitica* (Tauxe, 1987; Andersen, l988; Bissett, 1990; Hanna, 1988; Lee la, 1991). Infection with *Y. enterocolitica* can cause a variety of symptoms depending on the age of the person infected. Infection with *Y. enterocolitica* occurs most often in young children. Common symptoms in children are fever, abdominal pain, and diarrhea. Although infection can be due to contamination of milk or soy bean, but pork, especially chitterlings can be a source (Lee et al, 1991; Lee, 1990).

Receiving/Storage

For establishments processing raw products, ensuring that product entering the facility is not a source of microbial contamination can greatly reduce the probability and levels of contamination on outgoing product.

Raw products that are received as meat carcasses may be contaminated despite the usual step of steam pasteurization or hot water rinse (Phebus et al. 1997; Nutsch et al, 1998) applied prior to transfer. After steam rinse, carcasses are chilled (blast air chiller) for 24 to 48 hours. Fluctuations in chiller temperature, or the outright failure to adequately chill carcasses, may enable pathogen growth (Gill and Bryant 1997: Dorsa et al 1997).

Incoming meat could be also received in boxes from other facilities. Therefore, testing of product, or having purchasing specifications that require certification of product testing at the supplier, can help ensure that incoming bacterial loads are below those that can be handled by downstream controls.

Processing

Processing of raw products in this HACCP category involves a number of activities that present a high potential for cross-contamination, including mixing/grinding, formulating, needling, marinating, and rework.

Although the extent of bacterial contamination does not increase during the grinding process because of temperature controls, contaminated raw product from a single combo bin or box can be mixed with other raw product during grinding to contaminate many lots of product.

Ground product can be shaped into patties or packaged in bulk containers and shipped for consumption or further processed.

Further processing of raw ground product may include mixing, grinding, formulating (Riordan et al, 1998), needling, marinating and rework. Survival of microbes may differ depending on the meat mixture **(**Ahmed et al, 1995; Björkroth, 2005). Many of those activities result in extensive equipment contact with the raw product, creating opportunities for cross-contamination between the equipment and product (Rivas el al, 2004), and lot-to-lot contamination. Rework also can result in lot-to-lot contamination if not properly controlled. Maintaining temperatures cold enough to inhibit microbial growth and properly implementing sanitary procedures can greatly limit product contamination (Smith, 1985; 1987; Gill and Phillips, 1990).

Storage/Shipping

At storage/shipping, proper temperature is essential to control bacteria (Jackson et al, 1997; Gill, 1983; Scanga et al, 2000). Maintaining control of product (either holding it or not releasing it for sale to consumers) until any tests, by either FSIS, other government agencies, or the establishment, have been completed and shown to be negative, is an important control to protect public health.

Packaging/Labeling

Raw ground products should be labeled as to their intended use (e.g., For Cooking Only), and all ingredients need to be declared on the label. Failure to do either could represent a risk to the public downstream (Yang et al., 2000). Also, having product labeled to facilitate trace-back and trace-forward can control potential public health impacts.

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RAW NOT GROUND (03C)

Raw Not Ground meat and poultry products includes intact products, such as steaks and poultry parts (i.e., breast, wings), and products made with advanced meat recovery systems. Products in HACCP Category 03C should not have been marinated or water injected.

Pathogens of Concern

Salmonella is a pathogen of concern in raw meat products, and *Escherichia coli* O157:H7 represents a potential health hazard in beef products. *Salmonella* and *Campylobacter* are the primary pathogens of concern in poultry products. *Salmonella* is one of the most common causes of bacterial gastroenteritis in humans with approximately 40,000 cases of salmonellosis reported in the United States each year (Mead et al., 1999). Over 2,000 different *Salmonella* serotypes have been identified, and all have been determined to be pathogenic to humans (D'Aoust, 1997). Immuno-compromised individuals, such as children and the elderly, are the most at risk and more likely to suffer severe conditions from the symptoms associated with this organism. Although *E. coli* O157:H7 causes fewer reported illnesses when compared to *Salmonella*, the severity of the illness, with the development of hemolytic uremic syndrome, and the case mortality rate, particularly for immuno-compromised individuals, is significantly higher than for *Salmonella* (Mead et al., 1999). Because both *Salmonella* and *E. coli* O157:H7 are human pathogens and natural inhabitants of cattle and poultry, the presence of these organisms in cattle and poultry at slaughter and in associated products, poses a risk in raw beef and poultry products.

Receiving/Storage

For establishments producing raw products, ensuring that raw materials entering the facility are not a source of microbial contamination can greatly reduce the probability and levels of contamination on outgoing product. Testing of product, or having purchasing specifications that require certification of product testing at the supplier, can help ensure that incoming bacterial loads are below those that can be handled by downstream controls.

If the establishment is processing beef, it also should have controls in place related to Specified Risk Materials (SRM). Purchase requirements and checks at receiving need to be in place to make sure any SRMs are properly identified and destined only for acceptable use.

Proper temperature controls at the receiving and storage area also ensure that bacterial levels do not increase during storage. For example, to address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40°F or lower (FSIS, 2002; Barkocy-Gallagher, 2002).

Processing

The contamination of raw cuts of meat by pathogens such as *E. coli* O157:H7 and *Salmonella* spp. is primarily influenced by the bacteria on the carcass, parts, primals and trim. Processing of raw not ground products includes activities such as cutting and trimming and Advance Meat Recovery. Proper sanitation and temperature control during the processing step can reduce pathogen growth and cross-contamination of products.

During the cutting and deboning operations, contamination is possible from environmental sources and contaminated meat. The major source of contamination is likely to be the surface of incoming carcasses. Freshly cut surfaces of meat may be further contaminated when in contact with processing surfaces, equipment, conveyer belts, cutting surfaces, knives, gloves, and aprons during slaughter (Charlebois et al. 1991). Gill et al. (1999) found that despite a stringent sanitation regimen, and inspection by the national regulatory authority and internal plant quality assurance staff, *E. coli* O157:H7 persisted and proliferated on conveyer equipment in obscure areas that continued to contaminate the meat-contacting surface. Cross-contamination can occur via workers' hands and the commingling of trim (Newton et al. 1978). Fabrication rooms are typically kept at 10ºC (50ºF), but lapses may occur and the higher temperatures that result enable microbial growth.

Three studies report increases in general bacterial growth during this process. Hardin et al. (1995) report increased bacterial contamination on beef surfaces during the trimming process even with the use of sterile utensils under experimental conditions. Charlebois et al. (1991) sampled four locations within fabrication and concluded that the deboning operations resulted in the highest final count of fecal coliforms on boneless beef. A study in four plants found increases in generic *E. coli* contamination during fabrication ranging from 0 to 2 logs CFU/cm2 (Gill 1999).

Whole chickens carcasses also need to be deboned, parts trimmed and chopped in the cutting room. In large plants, breasts and thighs are commonly deboned with automated equipment. White meat, dark meat, and fat are collected separately. Hygiene is very strict, and cutting/deboning areas are kept at about 50 degrees F.

After cutting, trim is moved on conveyers to combo bins. If meat trim is cooled by dry ice in combo bins, microbial growth can be retarded (Gill 1996). However, Prasai et al. (1995) found no difference in concentrations of *E. coli* O157:H7 between hot deboning and cold deboning.
Numerous antimicrobials have been evaluated and approved as interventions for use on beef carcasses, primals, trim and ground products including lactic acid at 5%, acidified sodium chlorite, and more recently octanoic acid has been approved for fresh meat primals and subprimals when "applied to the surface of the product at a rate not exceed 400 ppm octanoic acid by weight of the final product" (USDA FSIS, 2007).

While many of these approved antimicrobials have been shown to be effective, either alone or in combination as multi-step hurdle approaches, most validations of the more current

antimicrobials are performed in-house (Bacon et al., 2000; Kang et al., 2001a; Kang et al., 2001b).

Packaging/Labeling

Raw products should be labeled as to their intended use (e.g., For Cooking Only), and all ingredients need to be declared on the label. In addition, meat processed using AMR needs to be correctly labeled. Failure to do either could represent a risk to the public downstream. Also, having product labeled to facilitate trace-back and trace-forward can control potential public health impacts.

Storage/Shipping

Temperature control (refrigeration) is a control measure most establishments have in place at this step for raw meat and poultry products in order to prevent growth of bacterial pathogens. To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40°F or lower (FSIS, 2002; Barkocy-Gallagher, 2002).

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THERMALLY PROCESSED, COMMERCIALLY STERILE (03D)

A 1971 finding of botulinum toxin in canned chicken vegetable soup and the death in 1974 of one person from botulism attributed to a product canned under USDA inspection prompted the revision of the canning regulations for meat and poultry. The new regulations were based on a HACCP concept - identifying critical control points setting critical limits, monitoring procedures, recordkeeping, and defining corrective actions for processing deviations or production errors, such as inadequate can seams.

Pathogens of Concern

From a public health perspective, the bacterial pathogens of most concern for these types of products are *Clostridium perfringens*, and *Clostridium botulinum* (Osherhoff et al, 1964), *Bacillus cereus cereus, Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus and Campylobacter jejuni* (Billon et al, 1977).

Clostridium botulinum, Clostridium perfringen, and *Bacillus cereus* are a concern for these meat and poultry products because of their ability to form spores (Guilfoyle et al, 1983; Barker et al, 1973; Odlaug and Pflug, 1978). Illnesses attributed to *Clostridium perfringens* annually ranks among the most common foodborne disease in Europe and the United States. CDC reported, for 1973 through 1987, that meat and poultry continued their traditional roles as the most common food vehicles for *Clostridium perfringens* type A food poisoning in the United States (Smith and Schaffner, 2004). Beef accounted for about 30% of all *C. perfringens* foodborne outbreaks, while turkey and chicken together accounted for another 15% of the outbreaks (Doyle et al., 1997)

Salmonella is one of the leading causes of bacterial foodborne disease outbreaks in the United States. Furthermore, most of the reported outbreaks are attributed to consumption of inadequately cooked, contaminated animal products (Goodfellow and Brown, 1978; Bean and Griffin, 1990; and Tauxe, 1991; Levine et al, 2001).

Listeria monocytogenes has been associated with numerous foodborne outbreaks worldwide. This bacterial pathogen accounts for 28% of the estimated foodborne deaths annually in the United States (Mead, 1999). For example, in 2002, there was a *Listeria monocytogenes* foodborne outbreak originating from fresh and frozen RTE chicken and turkey products that caused illness in more than 46 people, with seven deaths and three miscarriages (CDC, 2002). This bacterial pathogen is a significant public health concern for susceptible population groups such as pregnant women, the elderly, neonates, and immunocompromised individuals.

E. coli O157:H7 is also a public health concern for fully cooked, not shelf stable products made from beef and game animals (FSIS, 2004). First, it is well recognized that beef is a common source for the bacterial pathogen. Moreover, in recent years, several *E. coli* O157:H7 outbreaks have been linked to the consumption of undercooked ground beef patties (Clavero, 1998).

Receiving Raw Meat and Poultry

The raw meat and poultry used for the manufacture of fully cooked, shelf stable meat and poultry products are often contaminated with bacterial pathogens (e.g., *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7, *Clostridium perfringens*, and *Campylobacter jejuni*/*coli*) during the slaughter process (FSIS, 1994; FSIS, 1996; and FSIS, 1998).

Two control measures that an establishment may have in place at the receiving step include: (1) temperature control of incoming raw meat and poultry; and (2) purchase specifications for microbial levels. The purpose of the first control measure is to ensure that no bacterial pathogen growth occurs in raw meat and poultry during transit. The purpose of the second control measure is to ensure that the prevalence and level of bacterial pathogens on incoming source materials are low.

Receiving Non-meat/Non-poultry Food Ingredients

Non-meat or poultry ingredients include salt, sugar, spices, etc. which may contain pathogens and a high number of microorganisms per gram. Hara-Kudo et al. (2006) isolated two *Salmonella* spp. from black and red pepper (at least 1 CFU in 25 grams of sample). The aerobic bacterial count, a general indicator of sanitation, of garum masala, tumeric, curry powder and paprika was greater than 5.39 CFU/g. Vij et al. (2006) reported that there have been an increased number of recalls of dried spices due to bacterial contamination. Paprika was the most frequently involved in the recalls. Of 12 paprika recalls due to bacterial contamination, all but one was contaminated with *Salmonella*. These authors also noted that paprika contaminated with low numbers of *Salmonella* was the cause of a nationwide outbreak. *Bacillus cereus*, control of which is important in product cooling, is a common contaminant of spices (McKee, 1995).

Storage of Raw Meat and Poultry

Temperature control (refrigeration) is a control measure most establishments have in place at the storage step of raw meat and poultry in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7). Quite often, establishments address cold storage of raw meat and poultry in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40˚ F or lower (FSIS, 2002; and Barkocy-Gallagher, 2002).

Canned Meat or Poultry

The major reason for canning meat is to provide safe products that can be stored for long periods while preserving flavor, texture and appearance (Gilbert et al, 1982). The meat canning process presents some special considerations because they are low-acid foods. Microbes do not survive well in foods with high acid content.

FSIS defines a canned product as "a meat or poultry food product with a water activity above 0.85 which receives a thermal process either before or after being packed in a hermetically sealed container" (9 CFR 318.300 (d) and 9 CFR 381.300 (d)). These products will remain stable and retain their organoleptic quality for several years, even when held at room temperature. When most products are canned, they are treated with heat to make them shelf-stable (commercially sterile). FSIS considers shelf-stability and commercial sterility to be the same with respect to canning and canned products. The FSIS canning regulations define shelf-stability as "the condition achieved by application of heat, sufficient, alone or in combination with other ingredients and/or treatments, to render the product free of microorganisms capable of growing in the product at non-refrigerated conditions (over 50°F or 10°C) at which the product is intended to be held during distribution and storage. Shelf-stability and shelf-stable are synonymous with commercial sterility and commercially sterile, respectively" (9 CFR 318.300 (u) and 9 CFR 381.300 (u)).

Canned products are often referred to as "low-acid canned foods" (LACF) or "acidified low-acid foods," depending on whether the pH is above 4.6 (for LACF) or is 4.6 or below (for acidified foods). An acidified low-acid food is a canned product that has been formulated or treated (by addition of an acid or acid food) so that every component has a pH of 4.6 or below within 24 hours after the completion of the thermal process.

Commercially sterile uncured meat and poultry products include canned beef stew, whole chicken, chili, meat sauces and gravies, meat spreads, soups containing meat and poultry, baby and toddler foods, and even entrees such as chicken with noodles. Some of these, such as spaghetti sauce with meat, may be acidified products. Commercially sterile cured products include Vienna sausages, canned hams (not perishable), and canned luncheon meats.

Canning Process

The process of placing food in a container and heating it to make the product commercially sterile is known as conventional canning (Pearson and Gillett, 1999). To attain adequate safety standards, production of commercially sterile canned meat products requires that all viable microorganisms be either destroyed or rendered dormant. Commercially sterile canned meat products generally reach an internal temperature of at least 225^0F, but can be lowered to 215^0F providing that there is appropriate salt and nitrite content. This severe heat treatment can result in noticeable changes in flavor, texture and color. To better preserve product quality an alternative method of canning is by pasteurization. There is another method of producing commercially sterile products known as aseptic processing.

This method involves sterilizing the food and the container separately and filling and sealing the container in an aseptic (i.e., sterile) environment. This means of manufacturing commercially sterile products is not widely used for meat and poultry products in the U.S. at this time, although it is a proven technology. Other methods used in canning include high pressure processing.

Containers

Although commercially sterile products are referred to as "canned," the products may be in cans, glass jars, plastic containers, laminated pouches (Lebowitz, 1990), paperboard containers, etc., that are sealed to prevent the entry of microorganisms.

Various shapes of cans are used for canning meat. These include pear, oblong or round shaped, or Pullman-base cans of various heights. Different materials are presently used for cans. Tinplate cans are made of thin sheets of steel coated with a very thin film of tin. They can be anodized and enameled. To prevent interaction between the meat product and the metal, cans are generally coated on the inside with an organic material. These coatings, consisting of resins in organic solvents are referred to as lacquer or enamel. The coating for meat cans are sulfur resisting material which prevents the tinplate from staining black.

Some foods preserved in lacquer-coated cans and the liquid in them may acquire estrogenic activity. All estrogenic activity in these cans was due to bisphenol-A leached from the lacquer coating (Brotons et al, 1995). Therefore the use of plastic in food-packaging materials may require closer scrutiny to determine whether epoxy resins and polycarbonates contribute to human exposure to xenoestrogens.

Aluminum cans are also used although not used as extensively because they are more costly; however, they are lighter, resistant to sulfide and rust discolorations and easier to open. Aluminium foil is also an important material in laminates and has wide application in food packaging. With present toxicological knowledge, the use of aluminum in packaging material is considered to be safe, and inner-coating of the foil is recommended in specific cases.

Plastic is also used instead of cans. These containers can be flexible, such as pouches, or semi-rigid, as in lunch bowls (Berry and Bush, 1988).

Retorts

The most important phase of a sterile canning operation is retorting. This operation serves two purposes. First, products are subjected to sufficiently high temperatures to achieve destruction of all organisms that might adversely affect consumer health as well as those that could cause spoilage under storage conditions. Second, after retorting the product can be consumed directly out of the can without further cooking.

A retort is a steel tank in which metal crates or baskets containing the cans are placed for cooking and subsequent cooling. It is fitted with a cover or door, which can be closed to provide a seal the hold the cooking or cooling pressure. Three types of retorts are used in the food industry: (1) non-agitating ; (2) continuous agitating; and (3) hydrostatic.

Most canned meat products manufactured in the U.S. are cooked in non-agitating or stationery retorts. These retorts are closed-pressure vessels that operate in excess of atmospheric pressure and use pure steam or superheated water as the heating medium for cooking. Non-agitating retorts function on a batch basis in that the retort must be loaded, then closed, and the entire batch cooked before a second batch of product can be put in.

Federal regulations require samples of each processed lot be held at $95^0F \pm 2^0F$ for a minimum of 10 to 30 days before the cans leave the plant. One can must be incubated from each retort load. At the end of the incubation period, cans are examined for evidence of spoilage, as noted by end distortion of the cans prior to certification of the lot for discharge into the commerce.

Retort Schedule

To establish a retort schedule for a sterile canned meat product it is necessary to determine the rate of heat penetration at the slowest heating point in the can. This determination is done by fitting cans with needle-type thermocouples placed in the product and by means of a self-recording potentiometer, a temperature graph is obtained. From this information the lethal effect of a particular process is integrated with respect to the thermal death time of a specific microorganism. The Fo value for a process is an arbitrary value based on the destructive effect of the process on the viability of *Clostridium botulinum* of an equivalent number of minutes at 250^0F. In general, an Fo of 2.78 is considered to be a "botulinum safe cook". However, this process does not necessarily ensure freedom from spoilage by organisms that are more heat resistant then *Clostridium botulinum.*

The accuracy of the thermocouple device is critical to the process control. Depending on the type overestimation of process lethalities can occur (Zhang, 2002).

If performed according to Federal regulations, the retort process seldom results in a failed product. However, in 2007, several cases of botulism were found to be associated with commercially canned chili Sauce in Texas and Indiana. Examination of the canning facility in Georgia from which the cans originated identified deficiencies in the canning process (MMWR, 2007). The Castleberry's canning facility produces both FDA- and FSIS-regulated products. The outbreak investigation by FDA and FSIS identified production deficiencies that might have permitted spores of *C. botulinum* to survive the canning process.

Closing

Before closing the cans, large cuts of meat, such as hams and picnics, are pressed to ensure correct can fit and to eliminate air pockets.

Cooling

After the heating process, all canned meat products should be cooled rapidly to a level at which cooking and quality deterioration stop, and below the range at which any surviving thermophilic bacteria can grow. After final cooling, temperature in the product center should not exceed 100°F. The biological food safety hazards associated with cooling are *Clostridium botulinum*, *Clostridium perfringens,* and *Bacillus cereus*. These bacterial pathogens can form spores that survive the typical cooking process, which may subsequently germinate and multiply if held at abusive temperatures for too long. Consequently, it is very important that cooling be continuous through the given time/temperature control points (pre-established rates of time for temperature decline to meet specific temperatures during cooling). Excessive dwell time in the range of 130° to 80°F is especially hazardous, as this is the range of most rapid growth for the clostridia (Blankenship et al, 1988; Juneja et al, 1997; Smith and Schaffner, 2004). Therefore cooling between these temperature control points should be as rapid as possible.

When cans are being cooled, they contract and are subject to internal changes which may result in slight inward leakage into the even well-made cans. Therefore, canning cooling water is chlorinated. Sodium bisulfate can be also added to the water as a corrosion inhibitors.

For large diameter cans cooked in nonagitating retorts, cooling is done under pressure to prevent buckling of the can ends. Immersion biotesting has been used to challenge pack ages, particularly cans, for pinholes and channel leaks (Thompson, 1982).

The minimum channel leak size for shelf-stable poultry and meat products made in polymeric trays has been investigated and found to be 50-100 micros and can be used as a guide for pass and fail regulation parameters (Ravishankar et al, 2005).

Storage and Shelf Life

Canned products stored below 70°F should maintain acceptable palatability for 4 to 5 years. Pasteurized canned products stored below 40°F should maintain palatability for more than 2 years. The shelf life of hams processed in plastic cans is somewhat reduced (12-18 months).

Process failures in each of the steps involved in canning that resulted in growth of microbes associated with illness or spoilage has been reported in the literature. These include spoilage of product resulting from underprocessing, post-process leakage, contamination, or growth of thermophilic organisms, usually the result of storage at temperatures above 113°F. Underprocessing can be the result of inadequate time or temperature in retorting or poor control of a critical factor, such as pH. Post-process leakage contamination and thermophilic spoilage result from a break in the production process rather than failure in the process schedule. Can defects, such as dents, may affect the integrity of the can seams which may cause leaker spoilage. While thermophilic spoilage does not represent a potential health hazard, post-process leaker spoilage may result in the growth of gas-forming anaerobes, such as *C. botulinum*.

In addition to microbial spoilage, various physical and chemical contaminants may represent potential health hazards. For example, a product may be contaminated by a strong alkali from a cleaning solution. Physical hazards include, but are not limited to, glass in baby food jars, rubber from gaskets, and foreign objects or insecta not removed during cleaning prior to filling. Chemical hazards may involve strong alkali from cleaning solutions, heavy metals, or pesticides. Most of the physical and chemical hazards are introduced prior to filling and are not the result of processing. However, incidents of physical and chemical contamination are not well documented in the literature since reporting of such incidents is not required as are cases of most foodborne illnesses.

Pasteurized Canned Products

Pasteurized canned meats are cooked to an internal temperature of at least 150^{o}F. The reduced heat results in better preservation of flavor, texture, and color. However, the shelf life is usually much less than shelf-stable canned products. Therefore they must be labeled as perishable and must be kept refrigerated. The process does not result in complete destruction of all microbial contaminants (Roberts et al, 1981), but if properly executed, according to Federal inspection regulations, the product can be kept safe at least two years. Salt and nitrite present in the curing pickle also contribute significantly to the safety of pasteurized canned meats.

Federal regulations place the following restrictions on pasteurized products:

1). All products must be cured.
2). The net weight of each canned product must be 12 oz or greater.
3). Products must be cooked in cans to a center temperature of at least 150^{0}C.
4). Canned products must be labeled "Perishable—keep under refrigeration"
5). Canned products must be stored and distributed under refrigeration.

In addition, for hams, Federal regulations specify that the preparation for canning must not result in an increase of more than 8% in weight over the weight of fresh bone-in uncured hams.

Pasteurized canned meats are closed on a vacuum closing machine with 18 to 25 inches of machine vacuum.

Aseptic Canning

Asceptic canning was developed to improve finished product quality. The process involves sterilizing containers and products separately and then assembling them in an aseptic atmosphere to achieve a sterile package that can be stored at room temperature. The product is heated, while flowing continuously, to a temperature around 300^{o}F so that sterility can be achieved in a very short time. After cooling under sterile conditions sterile cans are filled within a sterile atmosphere.

Studies have shown that sterilization of meat separately adversely affect the quality of the finished product (Dawson et al, 1991). It is possible to reduce the toughening effect by presoaking the meat in a salt and sugar solution (Dawson, and Dawson, 1993).

High Pressure Processing (HPP)

In this process, cans are filled in a pressurized room under 18 lbs air pressure at a temperature of 225^{o}F and holding at this temperature for sufficient time to achieve sterilization. The cans are closed under the same conditions. When cans are closed with the product heated to around 255^{o}C, sterility is achieved by retaining the temperature for a few minutes and rapidly cooled. This process allows cans to be filled at a temperature that is not achievable under normal atmospheric conditions. The avoidance of prolonged cooking results in much greater preservation of product quality. By combining heat treatment of the product with application of high pressures, inactivation of spores and enzymes can be achieved. Recent studies suggest that currently used commercial high pressure processing parameters will effectively compromise and probably eliminate *C. jejuni* from pressure-processed foods given the pressure sensitivity of *C. jejuni* (Solomon and Hoover, 2004).

Overall, inactivation data of *C. botulinum* spores support the potential of HPP as a process, although variations in resistance among different spore populations seem to skew inactivation results (Rovere et al, 1998; Rodriguez et al, 2004; Black et al, 2007). Survival curves seem to depend highly on which strain and specific organism are targeted. Optimum levels of pressure and temperature need to be established to determine the most efficient and consistent kill rates.

FSIS Regulations

FSIS regulations for production of thermally processed, commercially sterile products are addressed in two subparts of the current regulations. The two sections are identical except that Subpart G, 318.300 – 311, pertains to meat products and Subpart X, 381.300 to 311, pertains to poultry products (FSIS). These regulations are either prescriptive, containing detailed requirements for containers and container closures, equipment specifications and operations, finished product inspection, product recall or overlap those of the HACCP – recordkeeping and record review, corrective actions in the event of a processing deviation, and implementation of validated process schedules.

Like processors of other RTE products, processors of thermally processed, commercially sterile meat and poultry products must address biological, physical, and chemical hazards when developing their HACCP plan. However, establishments do not have to address the food safety hazards associated with microbiological contamination if the product is produced according to the requirements in the meat or poultry canning regulations. This exception is contained in 417.2(b)(3) of the HACCP regulations. In permitting this exception, the Agency recognized that the canning regulations were "based on HACCP concepts and provide for the analysis of thermal processing systems and controls to exclude microbial hazards."

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NOT HEAT-TREATED, SHELF-STABLE (03E)

Based upon the existing scientific literature, not heat-treated, shelf-stable products are most vulnerable to bacterial pathogen survival, growth, and recontamination during the fermentation, heating, drying, and post-lethality (e.g., slicing and peeling) steps for dry/semi-dry fermented sausages, and during salting (cure contact time), post-salting (equalization), drying/ripening, and post-lethality steps for salt cured products. The literature also supports that the greatest opportunities for decreasing pathogen survival, growth, and recontamination are at the fermentation, heating, drying, salting, post-salting, drying/ripening, and post-lethality steps. The following sections provide a detailed description of the microbial hazards and possible control measure(s) present at each step in the not heat-treated, shelf-stable process.

Introduction

For the purposes of this discussion, not heat-treated, shelf-stable products are those from processes that do not apply heat as the primary lethality step. Not heat-treated, shelf-stable meat and poultry products consist of many diverse products (e.g., salt cured products – prosciutto, basturma, coppa, country cured hams— and dry/semi-dry fermented sausages – summer sausage, pepperoni, salami, soudjouk, Lebanon bologna. Depending how the product is processed, many of these products (e.g., country-cured ham, basturma, summer sausage, and pepperoni) can fall under more than one HACCP category.

The focus of this literature review is on the processing points where salt cured and fermented, not heat-treated, shelf-stable products are most vulnerable to bacterial pathogen survival, growth, and recontamination. Moreover, the vulnerabilities discussed for salt cured and fermented products also apply to the other not heat-treated—shelf-stable meat and poultry products.

From a public health perspective, the bacterial pathogens of most concern for these types of products are *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus*. For example, several *E. coli* O157:H7 foodborne outbreaks have been linked to dry fermented sausages (Naim et al., 2003). A *Salmonell*a outbreak in Pennsylvania was epidemiologically linked to the consumption of Lebanon bologna (Chikthimmah et al., 2001). *L. monocytogenes* has been detected in fermented sausage products before and after processing (Farber et al., 1988). Moreover, FSIS reported in 2001 that *L. monocytogenes* is the most frequently isolated pathogen of those included in the FSIS monitoring program for fermented sausages (Levine et al., 2001).

Clostridium botulinum and *Clostridium perfringens* are a concern for these types of meat and poultry products if they does not achieve shelf-stability or a low enough water activity (a_w) and/or pH to prevent the germination and outgrowth of those pathogens.

Receiving Raw Meat and Poultry

The raw meat and poultry used for the manufacture of not heat-treated, shelf-stable meat and poultry products (e.g., salami and pepperoni) are often contaminated with bacterial pathogens (e.g., *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7, *Clostridium perfringens*, and *Campylobacter jejuni*/*coli*) during the slaughter process (FSIS, 1994; FSIS, 1996; and FSIS, 1998). As stated earlier, the bacterial pathogens of most concern for these types of products are *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*.

Two control measures an establishment may have in place at the receiving which are not usually Critical Control Points (CCPs) in the HACCP plan are 1.) temperature control of incoming raw and poultry, and 2.) purchase specifications for microbial levels. The purpose of the first control measure is to ensure that no bacterial pathogen growth occurs in raw meat and poultry during transit. The purpose of the second control measure is to ensure that the prevalence and level of bacterial pathogens on incoming source materials are low.

For those not heat-treated, shelf-stable meat and poultry products that are ready-to-eat (RTE) and will be consumed without further cooking by the consumer, the selection of raw materials and the microbiological quality of raw meat become important control measures to help assure the safety of these RTE products (ICMSF, 2005). It is especially important to know the prevalence and level of bacterial pathogens, such as *Salmonella* spp. and *E. coli* O157:H7, on the raw meat and poultry if the fermented or salt-cured RTE process is not validated to achieve either a 6.5 $\log_{10}$ reduction or 7.0 $\log_{10}$ reduction of *Salmonella* in a not heat-treated, shelf-stable RTE meat and poultry product, respectively, and specifically achieve a 5.0 $\log_{10}$ reduction of *E. coli* O157:H7 in a not heat-treated, shelf-stable RTE that contains any amount of beef (FSIS, 2001).

Receiving Nonmeat/Nonpoultry Food Ingredients

Nonmeat or poultry ingredients (e.g., salt, sugar, and spices) may contain pathogens and a high number of microorganisms per gram. Hara-Kudo et al. (2006) isolated two *Salmonella* spp. from black and red pepper (at least 1 CFU in 25 grams of sample). The aerobic bacterial count, a general indicator of sanitation, of garum masala, tumeric, curry powder and paprika was greater than 5.39 CFU/g. Vij et al. (2006) reported that there have been an increased number of recalls of dried spices due to bacterial contamination. Paprika was the most frequently involved in the recalls. Of 12 recalls due to bacterial contamination, all but one was contaminated with *Salmonella*. These authors also noted that paprika contaminated with low numbers of *Salmonella* was the cause of a nationwide outbreak. *Bacillus cereus*, control of which is important in product cooling, is a common contaminant of spices (McKee, 1995).

Storage of Raw Meat and Poultry

Temperature control (refrigeration) is a measure most establishments have in place at the storage step of raw meat and poultry in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7). Quite often, establishments address cold storage of raw meat and poultry in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40°F or lower (FSIS, 2002; and Barkocy-Gallagher, 2002). The minimal growth temperatures for *Salmonella* and *E. coli* O157:H7 are just slightly above 40°F.

Processing

The early steps of processing often include one or more of the following procedures: tempering, block chipping, weighing, grinding, chopping, mixing, preparing casing, stuffing, forming, or rework. For salt cured products, such as country cured ham, the key steps for the microbial safety are the salting (cure contact time), post-salting (equalization), and drying/ripening steps. For dry/semi-drying fermented sausages, the key steps for microbial safety are the fermenting, heating, and drying steps. The key steps for the salt cured products and dry/semi-dry fermented sausages will be discussed below in their own section.

Temperature control (refrigeration) and/or short processing time are the control measures most establishments have in place during the processing step in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7) on product. Most of the time, establishments address temperature control (refrigeration) and/or short processing time in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40°F or lower (FSIS, 2002; Barkocy-Gallagher, 2002). Normally, failure to provide temperature control to prevent bacterial pathogen growth is not an issue during processing of dry and semi-dry fermented sausages. Cold product temperatures are necessary to ensure product quality during grinding/chopping when reducing meat to the desired particle size in order to ensure clean cutting of the meat particles and to minimize fat smears. During processing, lean meats are typically maintained at 28° to 30°F and fat meats at 27° to 28°F in order to produce dry/semi-dry fermented sausages with the desired product characteristics (Pearson and Tauber, 1984).

Rework: Rework is product that is partially processed or finished product that is then added back into the formulation at a rate of about 5 percent. The possibility exists that reworked product becomes contaminated from a food contact surface or bacterial growth occurs before the reworked product is added back into the formulation. For example, product could be exposed to a food contact surface contaminated with *L. monocytogenes* in the post

processing environment. If bacterial growth occurs before the rework is added back into the processing line, this could increase the bacterial load beyond that which the process is validated to eliminate. Bacterial growth can occur if product held for rework is maintained above 40°F for an extended period. Daskalov et al. (2006) assessed the effect of including contaminated rework in two cooked sausage formulations. The sausages containing inoculated emulsion, simulating contaminated rework, added to the product formulation showed a slightly greater number of surviving *L. monocytogenes* CFU/g after heating and after subsequent storage at 50°F than the sausages without inoculated emulsions.

Salting, Post-salting, and Drying Steps for Salt Cured-Shelf-stable Products:

For salt cured products, such as country-cured ham, many establishments will designate salting (cure contact time), post-salting (equalization), and drying/ripening steps as CCPs. However, there are some establishments that have addressed these key steps to reduce pathogens in a prerequisite program. The lethality of *Salmonella* and other pathogens achieved in a salt-cured product will depend on the interaction of salt content, pH, time and temperature of curing, cold smoking/drying and aging. These steps are necessary to prevent, eliminate, or reduce to an acceptable level the pathogens of concern- *Salmonella*, *Trichinella spiralis*, and *Listeria monocytogenes*. This combination of steps represents hurdles to bacterial growth since each step alone is not sufficient to meet the pathogen reduction requirements in an establishment's HACCP plan. The regulatory requirements in 9 CFR 318.10 for the elimination of trichinae from pork products may not eliminate the bacteria pathogens. The establishment's HACCP plan must address the bacterial pathogens of concern.

Cure Contact Time (Salting). During a dry salting, the ham is covered with a salt and cure mixture and held at 40°F for at least 28 days or no less than 1½ days per pound of ham (9 CFR 318.10). The time for salting phase for shelf-stable country cured hams is longer than it is for non-shelf-stable hams. The salting rapidly reduces the amount of water available for bacterial growth (i.e., decreases the water activity, a_w) (Reynolds et al., 2001) while the hold temperature (40°F) inhibits bacterial growth. (Leistner and Gould, 2002). If brine (salt in a water phase) is used instead of a dry salt-cure rub, it usually ranges from 60% to 70% of saturation (0.87 to 0.82 a_w) (Huang and Nip, 2001). A water activity below 0.93 will prevent the growth of most pathogens except *Staphylococcus aureus* (Farkas, 1997). Portocarrero et al. (2002a) concluded from their results that the higher salt content and lower a_w values on country-cured ham are important in controlling the growth of *S. aureus* and enterotoxin production. Moreover, it appears that staphylococcal enterotoxin production is inhibited at brine concentrations above 10%, especially when the pH is below 5.45 (Reynolds et al., 2001).

Equalization (Post-salting). The equalization phase is the time after the minimal cure contact time, removal of the excess salt, and before placement in the drying room. During the equalization period, the salt permeates to the inner tissues of the pork muscle. The concentration of salt with resulting decrease in water activity will inhibit the growth of bacteria during ripening (Leistner and Gould, 2002). This step is done under refrigeration (e.g., 40°F).

Drying/Ripening. From the work of Reynolds et al. (2001), it appears that most of the lethality for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* is achieved at this step. Portocarrero et al. (2002b) judged that a longer drying/ripening time to attain a lower a_w, such as that found with shelf-stable country cured hams, is needed to eliminate *L. monocytogenes*. They demonstrated that a cold smoke, smoking at a low temperature, was not sufficient to eliminate *L. monocytogenes* under their processing conditions but did provide a >6 $\log_{10}$ reduction of *L. monocytogenes*. In addition, the Portocarrero study (2002b) found that the level of *E. coli* O157:H7, which would not be expected in a ham, decreased faster than *Salmonella* or *L. monocytogenes*. They concluded that *Salmonella* and *E. coli* O157:H7 do not represent a potential health hazard in properly prepared country-cured hams but that *L. monocytogenes* does represent a potential problem. Reynolds et al. (2001) demonstrated a 5.0 $\log_{10}$ reduction of *Salmonella* and *E. coli* and that the proliferation of *S. aureus*, and hence enterotoxin production, was not a concern.

Fermenting, Heating, and Drying Steps for Shelf-stable Dry/Semi-dry Fermented Sausages

For dry/semi-dry fermented products, such as pepperoni, many establishments will designate the fermenting, heating, and drying steps as CCPs. However, there are some establishments that have addressed these key steps to reduce pathogens in a prerequisite program. The four pathogens associated with fermented sausage products are *S. aureus*, *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7. For *S. aureus*, the release of a heat stable enterotoxin after it has achieved a density of at least 10^5 CFU/g rather than the bacterium itself is responsible for foodborne illness. *E. coli* O157:H7 is a pathogen of concern in those products containing any amount of beef.

Fermentation, Drying and Ripening. Fermentation and drying ripening are two distinct steps in the process. The discussion of both is combined for clarity.

Growth of *S. aureus* is inhibited by the competitive growth of lactic acid bacteria, such as lactobacilli and pediococci (Hayman, 1982; Tatini, 1973). Large amounts of acid produced during longer fermentation should inhibit or reduce any *S. aureus*. In one study (Smith and Palumbo, 1978), a >6 $\log_{10}$ reduction of *S. aureus* was attributed to production of lactic acid. However, temperature abuse during fermentation or an excessive number of *S. aureus* initially, as has occurred when contaminated starter culture is used, may result in the substantial growth of *S. aureus* and the subsequent production of enterotoxin.

The degree-hours concept is the control measure used for this biological hazard (The American Meat Institute Foundation, 1995). Many establishments identify this control measure as a CCP in the HACCP plan. However, some establishments may address the degree-hours concept in a prerequisite program instead of as a CCP in the HACCP plan. In addition, there have been cases, where some establishments have *not* addressed the degree-hours concept at all in their HACCP system. In these cases, there is a significant public health concern.

Simply put, the degree-hours concept is the time, in hours, for the product to reach a pH ≤ 5.3 multiplied by the number of degrees the fermentation chamber is over 60°F (minimum growth temperature for S. aureus). The degree-hours is calculated for each temperature used during fermentation, but a constant chamber temperature may be used. The number of degree-hours is limited by the highest temperature in the fermentation process prior to reaching a pH of 5.3 or less. For example, if the highest chamber temperature is less than 90°F, the process is limited to fewer than 1,200 degree-hours; fewer than 1,000 degree-hours if the chamber temperature is between 90 and 100°F; or fewer than 900 degree-hours if the chamber temperature is greater than 100°F (The American Meat Institute Foundation, 1995).

Both *Salmonella* and *E. coli* O157:H7 have been isolated from fermented sausage products. The great variety of products and processing procedures hinder determining if an x-$\log_{10}$ reduction of one pathogen will always produce a y-$\log_{10}$ reduction of the other. This point is illustrated by two studies on the reduction of *Salmonella*, one in Lebanon bologna and the other in pepperoni. In a Lebanon bologna process (Smith et al., 1975b), a 3 to 4 $\log_{10}$ reduction of *Salmonella dublin* and a reduction of *Salmonella typhimurium* to undetectable levels was observed by the end of fermentation if starter culture was used. Little reduction in the numbers of salmonellae was observed if aged beef without starter culture was used. Similarly, Bacus (1997) noted that contamination of fermented meat products with *Salmonella* most likely results from an inadequate lactic acid production or a highly contaminated raw product. In addition, the Lebanon bologna study demonstrated the effect of different processes, with and without starter culture, on the reduction of *Salmonella* and the difference in reduction between two serotypes of the same organism. In a pepperoni process (Smith et al., 1975a), *Salmonella dublin* was detected after fermentation and subsequent 43 days of drying but *Salmonella typhimurium* was undetectable after 29 days of drying. The reduction of *S. dublin* and *typhimurium* occurred at different stages in the process for the Lebanon bologna and pepperoni products and *S. dublin* appeared more resistant to both fermentation and drying than S. *typhimurium* in both products.

Various studies have shown that fermentation and drying resulted in about a 2 $\log_{10}$ reduction of *E. coli* O157:H7 (Ellajosyula et al., 1998; Faith et al., 1997, Glass et al., 1992). Glass et al. (1992) reported that *E. coli* O157:H7 decreased by about 2 $\log_{10}$ CFU/g after fermentation, drying, and storage at 4°C for 6 weeks following the end of 18- to 21-day drying cycle for a fermented sausage formulation. However, a 5 to 6 $\log_{10}$ reduction of *E. coli* O157:H7 was observed in pepperoni sticks following fermentation, drying, and 2 weeks of storage at ambient temperature (21°C) (Faith et al., 1997). In one of the few studies that compared the combined effect of fermentation and drying on both *Salmonella*

and *E. coli* O157:H7, Ellajosyula et al. (1998) observed that the reduction of *Salmonella* and *E. coli* O157:H7 in Lebanon bologna was less than 2 $\log_{10}$ after fermentation to pH 4.7. In this study, *Salmonella* was equally or significantly ($P<0.01$) less resistant than *E. coli* O157:H7 to various combinations of pH levels achieved after fermentation and subsequent heating at 110°F to 120°F. Fermentation to pH 5.2 or 4.7 followed by heating at 110°F to 120° for specified times (e.g., 110°F for 20 hours or 120°F for 3 hours) resulted in >7 $\log_{10}$ reduction of both *Salmonella* and *E. coli* O157:H7. This study shows that a final heating step may be necessary to achieve the proposed $\log_{10}$ reduction of both *Salmonella* and *E. coli* O157:H7 in fermented sausage products.

The Blue Ribbon Task Force (Nickelson II et al., 1996) listed 5 options for achieving a 5D or equivalent inactivation of *E. coli* O157:H7. The listed options were (1) utilize a heat process as listed in Appendix A to the final rule "Performance Standards for the Production of Certain Meat and Poultry Products;" (2) include a validated 5D inactivation treatment; (3) conduct a "hold and test" program for finished product; (4) propose other approaches to assure at least a 5D inactivation; and (5) initiate a HACCP system that includes testing of raw batter and achieving at least a 2-$\log_{10}$ reduction of *E. coli* O157:H7. Option 1 refers to compliance guidelines used by industry for applying a heat treatment to achieve a 6.5 $\log_{10}$ reduction of *Salmonella* which may be too severe for some products, Options 3 and 5 involve testing of the finished product or ingredients, and are, therefore, dependent on the rigor of the testing program. Option 4 is an opportunity for industry or academia to validate processes that achieve a 5 log reduction of *E. coli* O157:H7. Option 2 was the intent of the Task Force research. The results from the Task Force studies indicated fermentation temperature, product diameter (55 or 105mm), and product pH were the determining factors in achieving a 5 $\log_{10}$ reduction of *E. coli* O157:H7. For example, at a pH ≥5.0 and an incubation temperature of 70°F, a heat treatment is needed regardless of product diameter. On the other hand, if the incubation temperature is 110°F, holding the product at incubation temperature would achieve at least a 5 $\log_{10}$ reduction of *E. coli* O157:H7 without an addition heat treatment for all diameter products and pH levels except 55mm sausage with a pH ≥5.0. (Note: the reduction is based on the average reduction achieved in the study minus 2 standard deviations.)

In addition, several research studies have shown that fermentation and drying were only sufficient to effect a 1- to 2-log reduction of *E. coli* O157:H7 in dry/semi-dry fermented sausages (Faith, 1998). Consequently, many dry/semi-dry fermented sausages, particularly in the U.S., have a significant "heat step" in the process to assure lethality of high numbers of bacterial pathogens. For example, in one study it was shown that the traditional nonthermal process for pepperoni was only sufficient to eliminate only low levels (≈2 log CFU/gram) of *E. coli* O157:H7. However, heating to internal temperature of 145°F instantaneous or 128°F for 60 minutes resulted in a 5 to 6 $\log_{10}$ reduction of the bacterial pathogen in pepperoni (Hinkens, 1996). In another study, it was shown that regardless of the target pH, fermentation alone resulted in only a 1.39 $\log_{10}$ reduction in *E. coli* O157:H7 in beef summer sausage. In contrast, fermenting the product to a pH of 5.0 then heating to an internal temperature of 130°F and holding for 30 or 60 minutes resulted in about a 5- or 7-log reduction, respectively, in *E. coli* O157:H7 (Calicioglu, 1997). Therefore, the heating step may be critical in achieving sufficient reduction of the pathogens of concern in

dry/semi-dry fermented sausage, and thus should be a critical control point, if used, in order to produce a safe RTE product.

Acid adaptation and acid tolerance to the lowered pH in fermented products also contributes to pathogen survival and must be considered when validating processes for fermented meat and poultry products. Acid tolerance and adaptation have been observed in both *Salmonella* and *E. coli* O157:H7. Tsai and Ingham (1997) reported that acid adaptation enhanced the survival of both *Salmonella* and *E. coli* O157:H7.

While some researchers observed only a 1 $\log_{10}$ decrease of *L. monocytogenes* during fermentation and drying (Johnson et al., 1988), others (Glass and Doyle, 1989) have observed a >4 $\log_{10}$ reduction. *L. monocytogenes* has been detected in fermented sausage products before and after processing (Farber et al., 1988). It is the most frequently isolated pathogen of those included in the FSIS monitoring program for fermented sausages. However, it is not known whether isolation of *L. monocytogenes* in the FSIS fermented sausage monitoring program resulted from environmental contamination, an inadequate process, or both. Despite its prevalence in fermented sausage products, no foodborne illnesses have been linked to *L. monocytogenes* in fermented sausages and only rarely for meat products in general. *L. monocytogenes* is not a reference organism for fermented sausages, however, the finding of *L. monocytogenes* in the finished product would result in regulatory action as provided for in the Agency's fermented sausage monitoring program.

Further Processing

For salt cured products, such as country-cured ham, the additional processing steps that may occur after the lethality steps discussed earlier, may include one or more of the following procedures: Slicing; and peeling. For fermented products, such as pepperoni, the additional processing steps that may occur after the lethality steps discussed earlier, may include one or more of the following procedures: boning; slicing; and cutting.

As for any post-lethality exposed RTE product, a major public health concern is the post-lethality contamination of the product by *Listeria monocytogenes* in the establishment environment. Most establishments will address the potential for post-processing contamination of RTE product by *Listeria monocytogenes* and other bacterial pathogens of concern in their hazard analysis by preventing it through their Sanitation SOPs or prerequisite program in order to justify that it is not a food safety hazard reasonably likely to occur. Ultimately, the effectiveness of their Sanitation SOPs or prerequisite program will determine whether or not this decision in their hazard analysis is valid.

Packaging/Labeling

As for any post-lethality exposed RTE product, a major public health concern is the post-lethality contamination of the product by *Listeria monocytogenes* in the establishment environment. Most establishments will address the potential for post-processing contamination of RTE product by *Listeria monocytogenes* and other bacterial pathogens of concern in their hazard analysis by preventing it through their Sanitation SOPs or prerequisite program in order to justify that it is not a food safety hazard reasonably likely to occur. Ultimately, the effectiveness of their Sanitation SOPs or prerequisite program will determine whether or not this decision in their hazard analysis is supportable.

There are two basic groups of not heat-treated, shelf-stable products: ready-to-eat (RTE) and not-ready-to-eat (NTRE) products. RTE products are those that have received a lethality treatment to eliminate pathogens and are edible without additional preparation, such as cooking for safety. In contrast, NRTE products require cooking for safety, before eating. Examples of not heat-treated—shelf-stable RTE products are prosciutto, salami, some basturma and country cured ham, some summer sausage and pepperoni, and Lebanon bologna.

The second class of not heat-treated, shelf-stable products are those that are NRTE. These may include country cured ham, dried chorizo, Chinese sausage, basturma, and soujouk. One hazard associated with these types of dried meats is that consumers often think due to the product's appearance that they are RTE and, as a result, fail to cook them. To add to the confusion, some chorizos, soujouk, and other typically NRTE sausages maybe fully processed and made RTE. Thus proper labeling is crucial for consumer protection. More specifically, the product's package should include the following conspicuous labeling features: Safe handling instructions, if product is not processed or marketed as a RTE product; terminology indicating that the product must be cooked for safety (e.g., Raw, Uncooked, or Cook Thoroughly), if it is not obvious that the product is raw; cooking and preparation instructions, validated to ensure food safety; and the nutrition facts, if present, should include a serving size based on the ready to cook reference amount (see Resource 1 of FSIS Directive 10, 240.4).

Finished Product Storage/Shipping

Generally, there are no biological, chemical, and physical food safety hazards that are reasonably likely to occur at the storage and shipping steps. Meat and poultry products with a water activity of 0.85 will assure safety from bacterial growth (*S. aureus* and *Listeria monocytogenes*). In addition, in accordance with the FSIS' food standards and labeling policy book, sausages are shelf-stable if they meeting following compositional factors:

- Dry sausage must have a Moisture Protein Ratio (MPR) of 1.9:1 or less, unless another MPR is cited under Moisture Protein Ratio in the Food Standards and Labeling Policy Book.
- Semi-dry—shelf-stable sausage must:
  - have an MPR ≤3.1 and a pH value of ≤5.0, or

- have an MPR ≤1.9 at any pH, or
- have a pH of ≤4.5 (or 4.6 with an a_w of ≤0.91) and internal brine concentration of ≥5% and must be intact (or vacuum packaged if sliced), cured, and smoked.

Research has shown that the USDA standards for shelf-stable dry and semi-dry sausages are conservative in term of pathogenic bacterial growth (i.e., *Staphylococcus aureus*) during vacuum-packaged storage at 21°C. Moreover, the studies clearly showed that *S. aureus* numbers decrease on fermented (pH ≤5.1) products with a wide range of salt concentrations and moisture content (Ingham et al., 2005). Therefore, it is critical that the establishment have documentation (e.g., published research, FSIS' Food Standards and Labeling Policy Book) to show that the salt cured and dry/semi dry fermented product is shelf-stable in order to ensure food safety.

Finished product storage is an additional intervention step that establishments can implement in order to further reduce bacterial pathogens on salt cured products and dry/semi-dry fermented sausages. For example, research has shown that counts of *S. aureus* decreased by 1.1 to 5.6 CFU for vacuum packaged summer sausages meeting and not meeting the USDA shelf-stability standard that were stored at 21°C for 4 weeks. Moreover, for three pepperoni products (two meeting the USDA MPR of ≤1.6, and one had a MPR of 1.7) it was shown that *S. aureus* decreased on the product by 3.0 to 4.5 log CFU after 1 week and was undetectable on two pepperoni products after 4 weeks. Similar results were seen for the six dried salami products that were studied (Ingham et al., 2005).

In another study, it was shown that the level of *Listeria monocytogenes* decreased by ≥ 1.0 log at room temperature (21˚ C) in one week for summer, elk summer, and buffalo summer sausage products. Consequently, the results suggest that summer sausage products with a mandatory 1-week predistribution storage period could be produced under alternative 1. However, processors must either ensure that their summer sausage had a_w and pH as low as those used in the research study or conduct a challenge study to validate the post-lethality treatment for their products (Ingham et al., 2004).

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HEAT-TREATED, SHELF-STABLE (03F)

Based upon the existing scientific literature on heat-treated, shelf-stable products are most vulnerable to bacterial pathogen survival, growth, and recontamination during the heat treatment, drying, and post-lethality (e.g., packaging) steps. The literature also supports that the greatest opportunities for decreasing pathogen survival, growth, and recontamination are at the processing, heat treatment, drying, and post-lethality steps. The following sections provide a detailed description of the microbial hazards and possible control measure(s) present at each step in the heat-treated—shelf-stable process.

Introduction

Heat-treated, shelf-stable meat and poultry products consist of many product types. Some examples are as follows: Lard, tallow, popped pork skins, bacon bits, some basturma, some summer sausage and pepperoni, biltong, soup mixes, beef nuggets, jerky, and snack sticks. Some of these products, such as basturma, summer sausage, and pepperoni can fall under more than one HACCP category depending how the product is processed.

Two of the most common heat-treated, shelf-stable products produced and consumed in the United States are jerky and snack sticks. The focus of this literature review is on the processing points where jerky is most vulnerable to bacterial pathogen survival, growth, and recontamination. In addition, vulnerabilities associated with the fermented snack sticks will be identified. Moreover, the vulnerabilities discussed for jerky and snack sticks also apply to the other heat-treated, shelf-stable meat and poultry products.

From a public health perspective, the bacterial pathogens of most concern for these types of products are *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus*. For example, at least eight gastroenteritis outbreaks were reported in New Mexico between 1966 and 1995 from ingestion of meat jerky. Two of these outbreaks were due to contamination with *Staphylococcus aureus* and six were due to contamination with *Salmonella* spp. (Eidson, 2000). Furthermore, FSIS reported in 2001 that the cumulative prevalence from 1990 to 1999 of *Salmonella* spp. and *Listeria monocytogenes* in jerky produced in federally inspected plants was 0.31 and 0.52%, respectively (Levine et al., 2001).

E. coli O157:H7 is also a public health concern for heat-treated, shelf-stable products made from beef and game animals (FSIS, 2004). First, it is well recognized that beef is a common source for the bacterial pathogen. Second, there has been a documented *E. coli* O157:H7 outbreak involving venison jerky (Keene, 1997).

Also, *Clostridium botulinum* and *Clostridium perfringens* are a concern for these types of meat and poultry products if the product does not achieve shelf-stability or a low enough water activity (a_w) and/or pH to prevent the germination and outgrowth of these two bacterial pathogens.

Finally, the two most important control steps for these types of meat and poultry products are heat treatment with sufficient humidity and drying to an appropriate low moisture content or a_w.

Receiving Raw Meat and Poultry

The raw meat and poultry used for the manufacture of heat-treated, shelf-stable meat and poultry products (e.g., jerky and snack sticks) are often contaminated with bacterial pathogens (e.g., *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7, *Clostridium perfringens*, and *Campylobacter jejuni*/*coli*) during the slaughter process (FSIS, 1994, 1996; 1998). As stated earlier, the bacterial pathogens of most concern for these types of products are *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*.

Two control measures that an establishment may have in place at the receiving step that are usually not Critical Control Points (CCPs) in the HACCP plan are 1.) temperature control of incoming raw and poultry and 2.) purchase specifications for microbial levels. The purpose of the first control measure is to ensure that no bacterial pathogen growth occurs in raw meat and poultry during transit. The purpose of the second control measure is to ensure that the prevalence and level of bacterial pathogens on incoming source materials are low.

For those heat-treated, shelf-stable meat and poultry products that are ready-to-eat (RTE) and will be eaten without further cooking by the consumer, the selection of raw materials and the microbiological quality of raw meat become important control measures to help assure the safety of these RTE products (ICMSF, 2005). It is especially important to know the prevalence and level of bacterial pathogens, such as *Salmonella* spp. and *E. coli* O157:H7, on the raw meat and poultry if the establishment is not relying upon Appendix A as a validated thermal process schedule. In addition, this is also the case, if the heat dried RTE process is not validated to achieve either a 6.5 $\log_{10}$ reduction or 7.0 $\log_{10}$ reduction of *Salmonella* in a heat-treated, shelf-stable RTE meat and poultry product, respectively, and specifically achieve a 5.0 $\log_{10}$ reduction of *E. coli* O157:H7 in a heat-treated, shelf-stable RTE that contains any amount of beef (FSIS, 2001).

Storage of Raw Meat and Poultry

Temperature control (refrigeration) is a control measure most establishments have in place at the storage step of raw meat and poultry in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7). Quite often, establishments address cold storage of raw meat and poultry in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40°F or lower (FSIS, 2002; Barkocy-Gallagher, 2002).

Processing

Processing often includes one or more of the following procedures: tempering; flaking; weighing, grinding; chopping; mixing; marinating; stuffing; tumbling; forming; fermenting; racking or hanging; or slicing. Temperature control (refrigeration) and/or short processing time are the control measures most establishments have in place during the processing step in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7) on product. Most of the time, establishments address temperature control (refrigeration) and/or short processing time in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40°F or lower (FSIS, 2002; Barkocy-Gallagher, 2002).

The use of antimicrobials during the marination step in conjunction with a heat step has been shown to increase the level of pathogen reduction above that achieved by heating alone (Calicioglu, 2002; Calicioglu, 2003; Albright, 2003). Some processes may not deliver an adequate lethality and, therefore, may require an additional intervention step to ensure product safety. Some establishments that use antimicrobials during the marination step may address the use of antimicrobials in a prerequisite program instead of as a CCP in the HACCP plan. Examples of such interventions are:

- Preheating the meat or poultry jerky strips in a marinade to a minimum internal temperature of 160°F provides an immediate reduction of *Salmonella*. However, since heating in the marinade may produce an unacceptable flavor for some products, other liquids, such as water, can be used. The times and temperatures in the lethality compliance guidelines (Appendix A) can be used for preheating in the liquid (Harrison and Harrison, 1996).

- Dipping the product in 5% acetic acid for 10 minutes before placing it in the marinade can augment the log reduction effects of drying but not enough to fully eliminate pathogens (Calicioglu et al., 2002, 2003; Albright et al., 2003).

- Dipping the product in 1% Tween 20 for 15 minutes and then into 5% acetic acid for 10 minutes followed by traditional marinade can augment the log reduction effects of drying but not enough to fully eliminate pathogens (Calicioglu et al., 2002, 2003; Albright et al., 2003).

For some heat-treated, shelf-stable meat and poultry products, such as some snack sticks, there is a fermentation step before the heat treatment step. The main microbial hazard associated with this fermentation step is *S. aureus* proliferation and the elaboration of its enterotoxins. The degree-hours concept is the control measure used for this biological hazard (The American Meat Institute Foundation, 1995). Many establishments identify this control measure as a CCP in the HACCP plan. However, some establishments may address the degree-hours concept in a prerequisite program instead of as a CCP in the HACCP

plan. In addition, there have been cases, where some establishments have *not* addressed the degree-hours concept at all in their HACCP system. In these cases, there is a significant public health concern.

Simply put, the degree-hours concept is the time, in hours, for the product to reach a pH ≤ 5.3 multiplied by the number of degrees the fermentation chamber is over 60°F (minimum growth temperature for S. aureus). The degree-hours is calculated for each temperature used during fermentation, but a constant chamber temperature may be used. The number of degree-hours is limited by the highest temperature in the fermentation process prior to reaching a pH of 5.3 or less. For example, if the highest chamber temperature is less than 90°F, the process is limited to fewer than 1200 degree-hours; fewer than 1000 degree-hours if the chamber temperature is between 90 and 100°F; or fewer than 900 degree-hours if the chamber temperature is greater than 100°F (The American Meat Institute Foundation, 1995).

Heat Treatment

The HACCP regulations require that establishments take measures to control, reduce, or eliminate the biological hazards identified in the hazard analysis. For meat and poultry jerky, these hazards will most likely include the microbiological hazards from *Salmonella spp.*, *Listeria monocytogenes*, and *Staphylococcus aureus*. For beef jerky, Escherichia coli O157:H7 may also be a hazard reasonably likely to occur. In recent years, several jerky products have been found to be adulterated with Salmonella and E. coli O157:H7 (Jerky Compliance Guidelines, 2004). While, most establishments have identified the heat treatment as a CCP in their Heat-treated, shelf-stable HACCP plans, many establishments have not identified humidity as a part of their heat treatment CCP's critical limit. Quite often, establishments address humidity in a prerequisite program or standard operating procedure (SOP) instead of as a part of the CCP's critical limit in the HACCP plan.

For meat jerky, use of the time-temperature combinations provided in the lethality compliance guidelines (Appendix A) would help to ensure the safety of the product. These time-temperature combinations are based on experiments that were done with ground beef without added salt or sugar. Added salt, sugar, or other substances that reduce water activity will increase the heat resistance of bacteria in a product. However, time and experience have shown that the time-temperature combinations in the lethality compliance guidelines are sufficient to produce safe products even those with both salt and sugar additives as long as adequate humidity is provided during heating.

For example, there was a reported *Salmonella* outbreak related to commercially produced beef jerky made in New Mexico that was contaminated with *Salmonella* Kiambu. The federally inspected establishment dried the jerky to a water activity of 0.3 or less in a dry 82°C (179.6°F) oven. Twenty percent of the jerky lots tested positive for *Salmonella*. However, the 82°C oven measured only 30°C (86°F) with a wet-bulb thermometer (Nummer et al., 2004).

In addition, several research studies have shown that the traditional drying process for jerky (10 h at 140°F) in a home style dehydrator is insufficient for destruction of pathogens in jerky (Nummer et al., 2004: and Harrison et al., 1997). For example, one study showed that the recommended 5-log reduction of *E. coli* O157:H7 was *not* achieved during 10 hours drying (air relative humidity 19-24%) of whole muscle beef jerky prepared without marinade and dried at 62.5°C (144.5°F), or prepared with marinade and dried for 10 hours at 62.5° C or at 68.3°C (154.9°F) (Albright et al., 2002). Generally, no moisture is added to these home style dehydrators and the air relative humidity is generally unknown.

In order to produce a safe, poultry jerky, producers can use the minimum internal temperatures listed in the lethality compliance guidelines of 160°F for uncured poultry or 155°F for cured poultry. They can also use the time-temperature combinations listed in the poultry time-temperature tables of the Draft Compliance Guidelines for Ready-To-Eat Meat and Poultry Products that are posted on the FSIS website www.fsis.usda.gov/OPPDE/rdad/FRPubs/Docs_97-013P.htm). However, humidity during heating is critical regardless of which compliance guideline is used. As with meat jerky, the time-temperature combinations would be sufficient to produce safe products with both salt and sugar additives if the processor uses the humidity parameters applicable to beef as described below.

Therefore, for both meat and poultry, the humidity parameters described for meat products must be followed if the lethality compliance guidelines are used as supporting documentation. The time-temperature tables are based on wet-heat. Without humidity the product will dry, and the bacteria will become more heat resistant (Goepfert, 1970; Goodfellow and Brown, 1978; Faith et al., 1998). As long as proper humidity is maintained, the level of pathogen reduction attained by using the lethality compliance guidelines for cooking poultry or whole beef should be sufficient to provide a safe product.

If the lethality compliance guidelines are used, the relative humidity must be maintained above 90 percent throughout the cooking or thermal heating process or by following one of the other humidity options specified in Appendix A. However, this level of humidity may not be necessary if an establishment can provide documentation that its process can achieve an adequate lethality with less humidity.

For example, research conducted at the University of Wisconsin, showed *Salmonella* reductions of ≥6.4 log CFU and similar reductions of *E. coli* O157:H7 were achieved by ensuring that high wet-bulb temperatures (125 or 130°F for 60 minutes, 135°F for 30 minutes, or 140°F for 10 minutes) were reached and maintained early in the process. For these wet-bulb spikes, 27, 32, 37, and 43% relative humidity, respectively, was obtained. After the completion of wet-bulb spikes, no further humidity was introduced into the smokehouse and the product was further dried at 170°F (dry-bulb temperature) (Buege, 2006).

The heating temperature and humidity (e.g., steam) are critical for achieving adequate lethality. As the water activity is reduced, the heat resistance (D value) of the bacteria increases (Goepfert, 1970). Therefore, if adequate humidity is not maintained during

heating, the time at a particular temperature to eliminate Salmonella will be greatly increased. It is crucial that the processor prevent drying of the product until a lethal time-temperature combination is attained. The humidity requirement must be applied during the first part of the heating process before any drying and an increase in solute concentration occurs.

The process should be monitored using wet and dry bulb thermometers as noted below (values in Appendix A are wet bulb product temperature values). The use of wet and dry bulb measurements can be used to determine relative humidity http://home.fuse.net/clymer/water/wet.html). For example, readings that show a difference of 2°F between the wet and dry bulbs might indicate approximately 94% relative humidity. Wet and dry bulb temperatures should not differ by more than 4.5°F. A temperature difference greater than 4.5°F indicates a relative humidity of approximately 86% and shows the needed minimum relative humidity (90%) is not being maintained.

At high altitudes, the amount of humidity in the chamber necessary to achieve a given log reduction of bacteria may need to be increased. Processing failures in the manufacture of jerky have occurred in establishments located at high altitudes.

Some simple and practical measures that can be used to help meet the humidity parameters in the lethality compliance guidelines are:

- **Seal the oven** - Close the oven dampers to provide a closed system and prevent moisture loss. Steam may be observed venting when the dampers are closed, similar to venting that occurs in a steam retort during canning.

- **Add humidity** - Place a shallow and wide pan of hot water in the oven to provide humidity in the system. Conduct a test run to determine whether the water evaporates. Injecting steam or a fine water mist in the oven can also add humidity. Use of a wet bulb thermometer, in addition to the dry bulb thermometer, also would enable the operator to determine if adequate humidity is being applied.

- **Monitor humidity** - Use a wet bulb thermometer in combination with a dry bulb thermometer. A basic wet bulb thermometer can be prepared by fitting a wet, moisture-wicking cloth around a dry bulb thermometer. To maintain a wet cloth during the process, submerse an end of the cloth in a water supply. The cloth must remain wet during the entire cooking step and should be changed daily, especially if smoke is applied. The use of a wet bulb thermometer is especially important for production at high altitudes or areas of low humidity where evaporation is facilitated.

Another vulnerability that can occur during the heat treatment step is significant growth of *S. aureus* when drying is not rapid and extends over a long period of time at temperature less than 60°C (140°F) (Holley, 1985). Normal levels in raw meat are usually 2 log/g, critical levels for human illness is more than 5 log/g, so conditions allowing more than 3

log growth would be of concern (ICMSF, 1996). The enterotoxins are very resistant to heat and would not be destroyed by Appendix A conditions (ICMSF 5, 1996).

Drying

Not all federally inspected establishments have addressed drying as a CCP in their heat-treated, shelf-stable HACCP plan. Some establishments have addressed drying in a prerequisite program instead of as a CCP in the HACCP plan. While some establishments address drying as a CCP with the drying temperature and time as the critical limits, others measure the water activity of the finished product as a HACCP verification procedure.

After the lethality treatment, the product should be dried to meet the MPR product standard of identity and to stabilize the finished product for food safety purposes and microbial stability. If the product is insufficiently dried, S. aureus and mold are potential hazards. These organisms should not grow in properly dried products. A suggested water activity (a_w) critical limit for stabilization of jerky is 0.80 or lower and vacuum-packing, or by drying and maintaining the a_w at $\leq$0.70 (ICMSF, 2005). This range of water activity should control growth of all bacterial pathogens of concern, as well as mold and yeasts.

The establishment should verify the water activity to demonstrate that the product has attained the critical limit for shelf-stability. Water activity is the key to determining the proper level of drying. The water activity can vary greatly at any given MPR (as a result of the presence and level of different solutes, such as sugar and salt) and highlights the problems associated with using MPR values to predict microbial growth (Ingham et al., 2006). Therefore, a laboratory test for water activity rather than total moisture should be used to verify proper drying.

A post-drying heat step is an additional intervention step that establishments can implement in order to further reduce bacterial pathogen population in either ground- or whole meat jerky strips. This involves heating the dried product in a 275°F oven for 10 minutes. This heating has the potential to reduce *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* levels by approximately 2 logs beyond the level of reduction achieved during initial heat step (Harrison et al., 2001; Nummer et al., 2004). This step may be needed for processes that do not achieve an adequate reduction of *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* during the heating process.

Packaging/Labeling

As for any post-lethality exposed RTE product, a major public health concern is the post-lethality contamination of the product by *Listeria monocytogenes* in the establishment environment. In addition, the association of jerky products with foodborne disease outbreaks have indicated the possibility that post-processing contamination by other bacterial pathogens (e.g., *E. coli* O157:H7 and *Salmonella*) could occur through cross-contamination of dried product with raw product via knives, work surfaces or through worker handling (Calicioglu, 2003). Most establishments will address the potential for post-processing contamination of RTE product by *Listeria monocytogenes* and other

bacterial pathogens of concern in their hazard analysis by preventing it through their Sanitation SOPs or prerequisite program in order to justify that it is not a food safety hazard reasonably likely to occur. Ultimately, the effectiveness of their Sanitation SOPs or prerequisite program will determine whether or not this decision in their hazard analysis is supportable.

There are two basic groups of dried meats: ready-to-eat (RTE) and not-ready-to-eat (NTRE) products. RTE products are those that have received a lethality treatment to eliminate pathogens and are edible without additional preparation, such as cooking for safety. In contrast, NRTE products require cooking before eating. The best known RTE dried meat is jerky. Other examples of heat-treated—shelf-stable RTE products are snack sticks, basturma, summer sausage, some pepperoni, lard, popped pork skins, and bacon bits.

The second class of dried meats are those that are NRTE. These may include dried beef, biltong, basturma, and soujouk. One hazard associated with these types of dried meats is that consumers often think due to the product's appearance that they are RTE and as a result, fail to cook them. To add to the confusion, some chorizos, soujouk, and other typically NRTE sausages are fully processed and made RTE. Thus proper labeling is crucial for consumer protection. More specifically the product's package should include the following conspicuous labeling features: Safe handling instructions, if product is not processed or marketed as a RTE product; terminology indicating that the product must be cooked for safety (e.g., Raw, Uncooked, or Cooked thoroughly), if it is not obvious that the product is raw; cooking and preparation instructions validated to ensure food safety; and the nutrition facts, if present, should include a serving size based on the ready to cook reference amount (see Resource 1 of FSIS Directive 10, 240.4).

Finished Product Storage/Shipping

Biological, chemical, and physical hazards are generally *not* food safety hazards reasonably likely to occur at the storage and shipping steps. Meat and poultry product with a water activity of 0.85 will assure safety from bacterial growth (*S. aureus* and *Listeria monocytogenes*). Consequently, if the establishment has documentation to show that the heat-treated meat and poultry product is shelf-stable this will assure food safety.

Chemical and physical hazards are not likely to occur at these steps since the product is usually packaged and boxed thus protecting it from any physical and chemical contamination.

Finished product storage is an additional intervention step that establishments can implement in order to further reduce bacterial pathogens on jerky and related products. For example, research has shown that counts of *S. aureus* decreased by 0.2 to 1.8 log CFU after 1 week of storage and by 0.6 to 5.3 log CFU after 4 weeks of storage at 21°C (69.8°F) for vacuum-packaged beef jerky. In addition, the research has shown that *Listeria monocytogenes* decreased by 0.6 to 4.7 log CFU after 1 week of storage and by 2.3 to 5.6 log CFU after 4 weeks of storage at 21°C (Ingham et al., 2006).

Also, research has shown that the use of antimicrobials in marinades used in jerky processing and the low water activity of the dried product provide antimicrobial effects for possible post-lethality contamination with *Listeria monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 (Calicioglu et al., 2003).

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FULLY COOKED, NOT SHELF-STABLE (03G)

Based upon the existing scientific literature for fully cooked, not shelf-stable, products are most vulnerable to bacterial pathogen survival, growth, and recontamination during the cooking, cooling, and post-lethality (e.g., slicing and peeling) steps. The literature also supports that the greatest opportunities for decreasing pathogen survival, growth, and recontamination are at the processing, cooking, cooling, and post-lethality steps. The following sections provide a detailed description of the microbial hazards and possible control measure(s) present at each step in the fully cooked, not shelf-stable processes

Introduction

Fully cooked, not shelf-stable meat and poultry products consist of many diverse products. Some examples are as follows: Cooked beef, roast beef, cooked corned beef products, fully cooked patties, beef barbecue, barbecued pork, frankfurter, frank, hotdog, wiener, Vienna bologna, garlic bologna, knockwurst, cheesefurters, and cooked ham.

The focus of this literature review is on the processing points where fully cooked, not shelf-stable meat and poultry products are most vulnerable to bacterial pathogen survival, growth, and recontamination.

From a public health perspective, the bacterial pathogens of most concern for these types of products are *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and *Clostridium botulinum* for cooked, perishable uncured meat and poultry. Moreover, for cooked, perishable cured meat and poultry products, the bacterial pathogens of most concern are *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*.

Salmonella is one of the leading causes of bacterial foodborne disease outbreaks in the United States. Furthermore, most of the reported outbreaks are attributed to consumption of inadequately cooked, contaminated animal products (Bean and Griffin, 1990; and Tauxe, 1991).

Listeria monocytogenes has been associated with numerous foodborne outbreaks worldwide. This bacterial pathogen accounts for 28% of the estimated foodborne deaths annually in the United States (Mead, 1999). For example, in 2002, there was a *Listeria monocytogenes* foodborne outbreak originating from fresh and frozen RTE chicken and turkey products that caused illness in more than 46 people, with seven deaths and three miscarriages (CDC, 2002). This bacterial pathogen is a significant public health concern for susceptible population groups such as pregnant women, the elderly, neonates, and immunocompromised individuals

E. coli O157:H7 is also a public health concern for fully cooked, not shelf-stable products made from beef and game animals (FSIS, 2004). First, it is well recognized that beef is a common source for the bacterial pathogen. Moreover, in recent years, several *E. coli*

O157:H7 outbreaks have been linked to the consumption of undercooked ground beef patties (Clavero, 1998).

Clostridium botulinum and *Clostridium perfringens* are also a concern for these types of meat and poultry products. *Clostridium perfringens* foodborne illness annually ranks among the most common foodborne disease in Europe and the United States. CDC reported, for 1973 through 1987, that meat and poultry continued their traditional roles as the most common food vehicles for *Clostridium perfringens* type A food poisoning in the United States. Beef accounted for about 30% of all *C. perfringens* foodborne outbreaks, while turkey and chicken together accounted for another 15% of the outbreaks (Doyle et al., 1997)

The most important control steps for these types of meat and poultry products are cooking, cooling, and sanitation/GMPs to prevent re-contamination of cooked products with *Listeria monocytogenes*.

Receiving Raw Meat and Poultry

The raw meat and poultry used for the manufacture of fully cooked, not shelf-stable meat and poultry products (e.g., hot dogs, roast beef, and cooked beef patties) are often contaminated with bacterial pathogens (e.g., *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7, *Clostridium perfringens*, and *Campylobacter jejuni*/*coli*) during the slaughter process (FSIS, 1994; FSIS, 1996; and FSIS, 1998). As stated earlier, the bacterial pathogens of most concern for these types of products are *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus, Bacillus cereus*, *Clostridium perfringens*, and *Clostridium botulinum*.

Two control measures that an establishment may have in place at the receiving step that are usually not Critical Control Points (CCPs) in the HACCP plan are: Temperature control of incoming raw and poultry; and purchase specifications for microbial levels. The purpose of the first control measure is to ensure that no bacterial pathogen growth occurs in raw meat and poultry during transit. The purpose of the second control measure is to ensure that the prevalence and level of bacterial pathogens on incoming source materials are low.

For those fully cooked, not shelf-stable meat and poultry products that are ready-to-eat (RTE) and will be eaten without further cooking by the consumer, the selection of raw materials and the microbiological quality of raw meat become important control measures to help assure the safety of these cooked RTE products (ICMSF, 2005). It is especially important to know the prevalence and level of bacterial pathogens, such as *Salmonella* spp., *Listeria monocytogenes*, and *E. coli* O157:H7, on the raw meat and poultry if the establishment is not relying upon Appendix A as a validated thermal process schedule. In addition, this is also the case, if the cooked RTE process is not validated to achieve either a 6.5 $\log_{10}$ reduction or 7.0 $\log_{10}$ reduction of *Salmonella* in a fully cooked-not shelf-stable RTE meat and poultry product, respectively (FSIS, 2001).

Receiving Nonmeat/Nonpoultry Food Ingredients

Nonmeat or poultry ingredients include salt, sugar, spices, etc. which may contain pathogens and a high number of microorganisms per gram. Hara-Kudo et al. (2006) isolated two *Salmonella* spp. from black and red pepper (at least 1 CFU in 25 grams of sample). The aerobic bacterial count, a general indicator of sanitation, of garum masala, tumeric, curry powder and paprika was greater than 5.39 CFU/g. Vij et al. (2006) reported that there have been an increased number of recalls of dried spices due to bacterial contamination. Paprika was the most frequently involved in the recalls. Of 12 paprika recalls due to bacterial contamination, all but one was contaminated with *Salmonella*. These authors also noted that paprika contaminated with low numbers of *Salmonella* was the cause of a nationwide outbreak. *Bacillus cereus*, control of which is important in product cooling, is a common contaminant of spices (McKee, 1995).

Storage of Raw Meat and Poultry

Temperature control (refrigeration) is a control measure most establishments have in place at the storage step of raw meat and poultry in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7). Quite often, establishments address cold storage of raw meat and poultry in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40˚ F or lower (FSIS, 2002; and Barkocy-Gallagher, 2002).

Processing

Processing often includes one or more of the following procedures: tempering; flaking; weighing, boning; trimming; dicing; grinding; chopping; emulsifying, mixing; mechanical tenderization; massaging; injecting; marinating; stuffing; tumbling; forming; racking or hanging; slicing; and rework. Temperature control (refrigeration) and/or short processing time are the control measures most establishments have in place during the processing step in order to prevent growth of bacterial pathogens (e.g., *Salmonella* and *E. coli* O157:H7) on product. Most of the time, establishments address temperature control (refrigeration) and/or short processing time in a prerequisite program instead of as a CCP in the HACCP plan.

To address the growth of most bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, it is usually recommended that raw meat and poultry be maintained at 40˚ F or lower (FSIS, 2002; Barkocy-Gallagher, 2002).

Addition of Lactates, Acetates, Diacetates (Antimicrobial Agents) to Meat Formulations

Studies have shown that lactic acid and acetic acid have significant antimicrobial activity in broth and food systems. Sodium and potassium salts of these acids, when added to processed meat formulations are also known to potentially inhibit pathogenic bacteria

especially L. monocytogenes. These antimicrobials inhibit growth of pathogens by inhibiting their metabolic activities. Interest in these antimicrobials is due to their ability to inhibit the growth of L. monocytogenes in post lethality exposed RTE meat and poultry products. Several studies of these antimicrobials have shown their ability to inhibit growth of *L. monocytogenes* in different meat formulations.

Rework

Rework is product that is partially processed or finished product that is then added back into the formulation at a rate of about 5 percent. The possibility exists that reworked product becomes contaminated from a food contact surface or bacterial growth occurs before the reworked product is added back into the formulation. For example, product could be exposed to a food contact surface contaminated with *L. monocytogenes* in the post-processing environment. If bacterial growth occurs before the rework is added back into the processing line, this could increase the bacterial load beyond that which the process is validated to eliminate. Bacterial growth can occur if product held for rework is maintained above 40°F for an extended period. Daskalov et al. (2006) assessed the effect of including contaminated rework in two cooked sausage formulations. The sausages containing inoculated emulsion, simulating contaminated rework, added to the product formulation showed a slightly greater number of surviving *L. monocytogenes* CFU/g after heating and after subsequent storage at 50°F than the sausages without inoculated emulsions.

Cooking

The HACCP regulations require that establishments take measures to control, reduce, or eliminate the biological hazards identified in the hazard analysis. For cooked, perishable meat and poultry products, these hazards will most likely include the microbiological hazards from Salmonella spp., Listeria monocytogenes, and Staphylococcus aureus. For cooked beef products, Escherichia coli O157:H7 may also be a hazard reasonably likely to occur. In recent years, several *E. coli* O157:H7 outbreaks have been linked to the consumption of undercooked ground beef patties (Clavero, 1998). While, most establishments have identified the cooking step as a CCP in their fully cooked, not shelf-stable HACCP plans, many establishments have not identified humidity as a part of their heat treatment CCP's critical limit. Quite often, establishments address humidity in a prerequisite program or standard operating procedure (SOP) instead of as a part of the CCP's critical limit in the HACCP plan.

For fully cooked, not shelf-stable meat products, use of the time-temperature combinations provided in the lethality compliance guidelines (Appendix A) would help to ensure the safety of the product. These time-temperature combinations are based on experiments that were done with ground beef without added salt or sugar. Added salt, sugar, or other substances that reduce water activity will increase the heat resistance of bacteria in a product. However, time and experience have shown that the time-temperature combinations in the lethality compliance guidelines are sufficient to produce safe products even those with both salt and sugar additives as long as adequate humidity is provided

during heating. Furthermore, the time-temperature combinations in the lethality compliance guidelines do *not* take into account the additional lethality that occur during the cooking come up time and come down time for fully cooked, not shelf-stable meat products.

In order to produce a safe fully cooked, not shelf-stable poultry product, processors can use the minimum internal temperatures listed in the lethality compliance guidelines of 160°F for uncured poultry or 155°F for cured poultry. They can also use the time-temperature combinations listed in the poultry time-temperature tables of the Draft Compliance Guidelines for Ready-To-Eat Meat and Poultry Products that are posted on the FSIS website (www.fsis.usda.gov/OPPDE/rdad/FRPubs/Docs_97-013P.htm). However, humidity during heating is critical regardless of which compliance guideline is used. As with fully cooked, not shelf-stable meat products, the time-temperature combinations would be sufficient to produce safe products with both salt and sugar additives if the processor uses the humidity parameters applicable to beef as described below.

For both fully cooked, not shelf-stable meat and poultry, the humidity parameters described for meat products must be followed if the lethality compliance guidelines are used as supporting documentation. The time-temperature tables are based on wet-heat. Without humidity the product will dry, and the bacteria will become more heat resistant (Goepfert, 1970; Goodfellow and Brown, 1978; Faith, N.G. et al. 1998). As long as proper humidity is maintained, the level of pathogen reduction attained by using the lethality compliance guidelines for cooking poultry or whole beef should be sufficient to provide a safe product.

If the lethality compliance guidelines are used, the relative humidity must be maintained above 90 percent throughout the cooking or thermal heating process or by following one of the other humidity options specified in Appendix A. However, this level of humidity may not be necessary if an establishment can provide documentation that its process can achieve an adequate lethality with less humidity.

The heating temperature and humidity (e.g., steam) are critical for achieving adequate lethality. As the water activity is reduced, the heat resistance (D value) of the bacteria increases (Goepfert, 1970). Therefore, if adequate humidity is not maintained during cooking, the time at a particular temperature to eliminate Salmonella will be greatly increased. It is crucial that the processor prevents drying of the product surface until a lethal time-temperature combination is attained. The humidity requirement must be maintained during cooking in order to prevent drying of the product's surface and an increase in solute concentration occurs.

The process should be monitored using wet and dry bulb thermometers as noted below (values in Appendix A are wet bulb product temperature values). The use of wet and dry bulb measurements can be used to determine relative humidity (http://home.fuse.net/clymer/water/wet.html). For example, readings that show a difference of 2°F between the wet and dry bulbs might indicate approximately 94% relative humidity. Wet and dry bulb temperatures should not differ by more than 4.5°F. A temperature difference greater than 4.5°F indicates a relative humidity of approximately 86% and shows the needed minimum relative humidity (90%) is not being maintained.

At high altitudes, the amount of humidity in the chamber necessary to achieve a given log reduction of bacteria may need to be increased. Processing failures in the manufacture of jerky have occurred in establishments located at high altitudes.

Some simple and practical measures that can be used to help meet the humidity parameters in the lethality compliance guidelines are:

- **Seal the oven** - Close the oven dampers to provide a closed system and prevent moisture loss. Steam may be observed venting when the dampers are closed, similar to venting that occurs in a steam retort during canning.

- **Add humidity** - Place a shallow and wide pan of hot water in the oven to provide humidity in the system. Conduct a test run to determine whether the water evaporates. Injecting steam or a fine water mist in the oven can also add humidity. Use of a wet bulb thermometer, in addition to the dry bulb thermometer, also would enable the operator to determine if adequate humidity is being applied.

- **Monitor humidity** - Use a wet bulb thermometer in combination with a dry bulb thermometer. A basic wet bulb thermometer can be prepared by fitting a wet, moisture-wicking cloth around a dry bulb thermometer. To maintain a wet cloth during the process, submerse an end of the cloth in a water supply. The cloth must remain wet during the entire cooking step and should be changed daily, especially if smoke is applied. The use of a wet bulb thermometer is especially important for production at high altitudes or areas of low humidity where evaporation is facilitated.

Another vulnerability that can occur during the cooking step is significant growth of *S. aureus* during slow cooking come up time. A cooking dwell time of greater than 6 hours in the 50˚F to 130˚F range should be viewed as especially hazardous, as this temperature range can foster substantial growth of many bacterial pathogens of concern (FSIS, 1999). The normal *S. aureus* levels in raw meat are usually 2 log/gram, critical levels for human illness is more than 5 log/gram, so conditions allowing more than 3 log growth would be of concern (ICMSF, 1996). The enterotoxins are very resistant to heat and would not be destroyed by Appendix A conditions (ICMSF 5, 1996).

Cooling

The biological food safety hazards associated with cooling are *Clostridium botulinum*, *Clostridium perfringens,* and *Bacillus cereus*. These bacterial pathogens can form spores that survive the typical cooking process, which may subsequently germinate and multiply if held at abusive temperatures for too long. Consequently, it is very important that cooling be continuous through the given time/temperature control points (pre-established rates of time for temperature decline to meet specific temperatures during cooling). Excessive dwell time in the range of 130° to 80°F is especially hazardous, as this is the range of most rapid

growth for the clostridia. Therefore cooling between these temperature control points should be as rapid as possible.

Not all federally inspected establishments have addressed cooling as a CCP in their fully cooked, not shelf-stable HACCP plan. Some establishments have addressed cooling in a prerequisite program instead of as a CCP in the HACCP plan. In addition, there are establishments that do not monitor that each lot of cooked, perishable meat and product is cooled to a low temperature within a certain time frame, but instead assume that the product will be cooled down within a certain time frame based on certain operating conditions.

Listed below are the cooling guidelines from the draft Compliance Guidelines or the Processing of Ready-to-Eat Meat and Poultry Products. These three cooling guidelines are very similar to the cooling guidelines listed in Appendix B, but with some modifications. FSIS considers these guidelines, if followed precisely, to be validated process schedules, since they contain processing methods already accepted by the Agency as effective.

1. During cooling, the product's maximum internal temperature should not remain between 130°F and 80°F for more than 2.0 hours or between 80°F and 40°F for more than 5 hours. This cooling rate can be applied universally to cooked products (e.g., partially cooked or fully cooked, intact or non-intact, meat or poultry) and is preferable to guideline #2 below.

2. FSIS has traditionally permitted product to be cooled according to the following procedures which were intended to assure no more than one log relative growth of C. perfringens: Chilling should begin within 90 minutes after the cooking cycle is completed. All product should be chilled from 120°F (48°C) to 55°F (12.7°C) in no more than 6 hours. Chilling should then continue until the product reaches 40°F (4.4°C); the product should not be shipped until it reaches 40°F (4.4°C).

   If an establishment uses this older cooling guideline it should ensure that cooling is as rapid as possible, especially between 130°F and 80°F, and monitor the cooling closely to prevent deviation. If product remains between 130°F and 80°F more than two hours, compliance with the new performance standards is less certain.

3. The following process may be used for the slow cooling of ready-to-eat meat and poultry cured with nitrite. Products cured with a minimum of 100 ppm ingoing sodium nitrite and a minimum brine concentration of 4.0% may be cooled so that the maximum internal temperature is reduced from 130°F to 80°F in 5 hours and from 80°F to 45°F in 10 hours (15 hours total cooling time). The 4% brine concentration is the biggest change from the Appendix B dated June 1999. The 4% brine concentration was added in order to prevent the germination of Clostridium botulinum. This was an oversight in the original Appendix B.

Establishments that incorporate a “pasteurization” treatment (one that does not achieve a full cook) after lethality and stabilization treatments (e.g., applying heat to the surface of a cooled ready-to-eat product after slicing), and then re-stabilize (cool) the product, should

assess the cumulative growth of C. perfringens in their HACCP plans. That is, the entire process should allow no more than 2-$\log_{10}$ total growth of C. perfringens or no more than 500 C. perfringens CFU per gram in the finished product before shipment. When employing a post-processing "pasteurization," establishments may want to keep in mind that at temperatures of 130°F or greater, C. perfringens will not grow.

Another vulnerability that can occur during cooling is how establishments handle cooling deviations or cooling unforeseen hazards. Many federally inspected establishments are currently using the Agricultural Research Service (ARS) Pathogen Modeling Program (PMP) *Clostridium perfringens* cooling model for beef broth without validating the model for their cooked, uncured meat and poultry products. Research has shown that this cooling model under predicts *Clostridium perfringens* growth at intermediate observed increases (1 to 3 logs CFU/ml) (Smith et al., 2004). Consequently, establishments may make an erroneous disposition decision for product that has experienced a cooling deviation. The Microbiology Division, Office of Public Health Science, has personally dealt with several cases where this has occurred.

Packaging/Labeling

As for any post-lethality exposed RTE product, a major public health concern is the post-lethality contamination of the product by *Listeria monocytogenes* in the establishment's environment. Most establishments will state in their hazard analysis that the potential hazard of post-processing contamination of RTE product by *Listeria monocytogenes* and other bacterial pathogens of concern is prevented by their Sanitation SOPs or prerequisite program in order to justify that it is not a food safety hazard reasonably likely to occur. Ultimately, the effectiveness of their Sanitation SOPs or prerequisite program will determine whether or not this decision in their hazard analysis is supportable. Discussed below are some of the microbial interventions that can be implemented before and after packaging in order to address any post-lethality contamination of product by *L. monocytogenes*.

Post-Lethality Treatment

Post lethality treatments such as steam pasteurization, hot water pasteurization, radiant heating and high pressure processing have been developed to prevent or eliminate post-processing contamination by L. monocytogenes. RTE products where post-lethality treatments were shown by studies to be effective in reducing the level of L. monocytogenes are whole or formed ham, whole and split roast beef, turkey ham, chicken breast fillets and strips, and sliced ham, sliced turkey, and sliced roast beef (FSIS, 2006).

Post-lethality treatments can be applied as a pre-packaging treatment, e.g. radiant heating, or as post-packaging treatments, e.g., hot water pasteurization, steam pasteurization, and high pressure processing. Ultra violet treatment can be used either as a post-lethality treatment or antimicrobial agent or process depending on whether it eliminates, reduces or suppresses growth of L. monocytogenes. Some of the published studies on post-lethality treatments are reviewed in Attachment 4 of the Listeria monocytogenes compliance

guidelines. Studies on post-lethality treatments showed reductions of inoculated L. monocytogenes from 1 to 7 log10 CFU/g depending on the product type, and duration, temperature and pressure of treatment. Higher log reductions were obtained when both pre-packaging and post-packaging surface pasteurizations were applied, and when post-lethality pasteurization was combined with the use of antimicrobial agents. Establishments should refer to the details of these studies if they want to use the intervention method in their processing. The guidelines will be updated to include studies or other methods as they become available (FSIS, 2006).

A pre-packaging treatment such as radiant heating can be used as a post-lethality treatment as long as it is validated to eliminate or reduce the level of L. monocytogenes. Since this is a post-lethality pre-packaging treatment, there is possible exposure to the environment after the treatment and before packaging. If there is separation between the treatment and packaging, then conditions have to be met to ensure a hygienic environment to preclude contamination, or the post-lethality treatment would not likely be considered effective by FSIS. Some establishments may place the packaging machine right after the radiant heat treatment to reduce or eliminate this exposure. Support documentation must be made a part of the hazard analysis decision-making documents and validation data must be included in the HACCP plan. Studies have also shown that the use of pre-packaging treatment combined with a post-lethality treatment resulted in a higher log reduction of the pathogen (FSIS, 2006).

Ready-to-Eat (RTE) versus Not Ready-to-Eat (NRTE)

It is possible that a company that makes a fully cooked product could market and label it as a not-ready-to-eat (NRTE) product and be exempt from the Agency's microbiological sampling program for RTE products provided that there is no product standard of identity that has identified the particular product as a cooked (e.g., hotdogs) or RTE product or that it is understood by consumers to be a RTE product (e.g., soups, stews, chili, and corned beef hash). For example, a company may contend that they heat the product, for quality (flavor, texture, etc.) purposes rather than to eliminate a biological hazard and that they expect that the consumer will fully cook the product and eliminate any pathogens of concern at that point, prior to eating. In these situations, we would expect that the company's HACCP plan support this contention (i.e., they have not identified a biological hazard such as Salmonella spp., E. coli O157:H7, Listeria monocytogenes, and Staphylococcus aureus at this point in their process that they are then eliminating with a subsequent heat (lethality) step). Consequently, a key thing that should be done is to determine how the preparation of the product is addressed in the HACCP plan's hazard analysis. The process, hazard analysis, HACCP plan and decision-making documents must be consistent with the manner the company chooses to label and market the product.

As discussed above, there, are two basic groups of cooked, perishable meat and poultry products: ready-to-eat (RTE) and not-ready-to-eat (NTRE) products. RTE products are those that have received a lethality treatment to eliminate pathogens and are edible without additional preparation, such as cooking for safety. In contrast, NRTE products require cooking before eating. A well-known cooked, perishable RTE meat and poultry product is

hotdogs. Other examples of fully cooked, not shelf-stable RTE products are stews, chili, soups, frankfurter, frank, Vienna bologna, garlic bologna, knockwurst, and corned beef hash.

The second class of cooked, perishable meat and poultry products are those that are NRTE. NRTE products are those that have not received a lethality treatment to eliminate pathogens and require additional preparation, such as cooking for safety. One hazard associated with these types of cooked, perishable meat and poultry products is that consumers often think due to the product's appearance that they are RTE and as a result, fail to cook them. Thus, proper labeling is crucial for consumer protection. More specifically the product's package should include the following conspicuous labeling features: Safe handling instructions, if product is not processed or marketed as a RTE product; terminology indicating that the product must be cooked for safety (e.g., Raw, Uncooked, or Cooked thoroughly), if it is not obvious that the product is raw; cooking and preparation instructions validated to ensure food safety; and the nutrition facts, if present, should include a serving size based on the ready to cook reference amount (See Resource 1 of FSIS Directive 10, 240.4).

Finished Product Storage/Shipping

Growth of *Listeria monocytogenes* is a potential biological hazard at the storage and shipping step. If post-lethality contamination occurs, the lower growth limit for *L. monocytogenes* is about 32°F. Although growth is very slow at this temperature, with generation times of 62 to 131 hours (ICMSF, 1996), over a period of weeks, there may be significant growth of this bacterial pathogen. Consequently, some of the control measures that may be implemented to prevent or limit the growth of *Listeria monocytogenes* are frozen storage, growth inhibitor packaging, and the addition of antimicrobial agents (e.g., acetate/lactate).

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HEAT-TREATED, NOT FULLY-COOKED, NOT SHELF-STABLE (03H)

Partially cooked beef patties and are examples of heat-treated, not fully cooked meat and poultry products that are not shelf-stable. Products in this category receive a thermal process that is insufficient to eliminate pathogens. These products receive a minimum thermal process or cold smoke. The thermal process requires that the product be properly cooled to prevent the growth of pathogens. The surface of the product may appear cooked. The cooked appearance can be from char-marks on a meat or poultry patty or breading that is browned or darkened. A description of not ready-to-eat (RTE), not shelf-stable products is located on the Food Safety and Inspection Service website at: http://www.fsis.usda.gov/PDF/RTE_Process_Familiarization.pdf. Examples of products in this category included partially cooked meat patties, breaded poultry, and bacon. are ready to cook poultry, cold smoked and products smoked for trichinae, partially cooked battered, breaded, char-marked, batter set, and low temperature rendered products, etc.

Information from the Scientific Literature

The steps in the process cited are those cited in the FSIS Generic HACCP Model for Heat-treated But Not Fully Cooked, Not Shelf-stable Meat and Poultry Products. The steps listed below include both control points and critical control points in the process for microbiological food safety hazards only. The critical control points (CCPs) are those points or steps in the process at which control or action can be applied to eliminate, prevent, or reduce a food safety hazard to an acceptable level. The control points are those steps to control a potential food safety hazard but which will not result in the elimination, prevention, or reduction of the food safety hazard. However, control points can reduce the hazard and critical limit that must be applied at the critical control point. If growth or contamination is not controlled at these points, the level of pathogens may exceed the level of reduction needed and which the validated process is designed to achieve.

Receiving Raw Meat and Poultry

The temperature of the in-coming meat and poultry must be maintained below that allowing growth of pathogens. *Salmonella* is a pathogen of concern in raw meat products, and *Escherichia coli* O157:H7 represents a potential health hazard in beef products. *Salmonella* and *Campylobacter* are the primary pathogens of concern in poultry products. If the temperature of the product is not maintained at or below 40°F, these pathogens can growth. The optimum temperature growth ranges for *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 are 95-109.4°F, 107.6-109.4°F, and 95-104°F, respectively (T.A. Roberts et al., 1996). However, *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 can grow, albeit slowly, at temperatures of 41.4°F, 89.6°F, and 44.6-46.4°F, respectively. *E. coli* O157:H7 can grow rapidly at 50°F.

Receiving Nonmeat/Nonpoultry Food Ingredients:

Nonmeat and nonpoultry ingredients include salt, sugar, spices, etc. which may contain pathogens and a high number of microorganisms per gram. Vij et al. (2006) reported that there have been an increased number of recalls of dried spices due to bacterial contamination. Paprika was the most frequently involved in the recalls. Of 12 recalls due to bacterial contamination, all but one was contaminated with *Salmonella*. These authors also noted that paprika contaminated with low numbers of *Salmonella* was the cause of a nationwide outbreak. In a review of publications on microbial contamination of spices and herbs, McKee (1995) cited a report from the United States in which *C. perfringens* was identified in four spices and was considered to be a health hazard, emphasizing the importance for rigorous standards of cleanliness of spices. Other reports cited by McKee indicated varying levels of *C. perfringens*. *Bacillus cereus*, which is a potential hazard in an improperly cooled product, was a common contaminant of spices (McKee, 1995).

Storage (Frozen/Refrigerated) Raw Meat and Poultry

The same reasoning for receiving raw meat and poultry applies to storage of these products. As noted above on receiving raw material, temperatures above 40°F will permit slow growth of pathogens. The minimal growth temperatures for *Salmonella* and *E. coli* O157:H7 are only slightly above 40°F.

Tempering Frozen Meat and Poultry

The tempering of frozen meat and poultry can be conducted in cold running water or microwave designed for tempering. The temperature of the meat surface should not rise above common holding temperatures for extended periods of time to prevent the outgrowth of bacterial pathogens.

Mechanical Process:

The surface of the whole muscle meat or poultry product is contaminated on the surface but the interior should be free of contamination if the whole muscle was handled properly. During deboning, mixing, or stuffing any surface contamination is moved into the interior of the product. Mixing may create a uniform distribution of bacteria within the product but the distribution of bacteria is probably not uniform in a deboned or stuffed product. Injecting meat or poultry can force the surface bacteria to the product interior. The needles, same as blades, used in injecting can carry a surface contaminant to the product interior.

During mechanical tenderization, the blades or needles can transfer microorganisms from the surface of the meat to the interior (Johnston et al., 1978; Gill and McGinnis, 2004; Gill et al., 2005; Sporing, 1999). Sporing (1999) demonstrated that 3-4% of a surface inoculum of *E. coli* O157:H7 was translocated to the center of the product.

The solution injected into the meat or poultry could also be the source of contamination. In 2003, the cases of foodborne illness were linked to mechanically tenderized and injected

steaks produced at a federally inspected processing plant and sold door-to-door (Laine et al., 2005). The steaks in the 2003 outbreak were injected with a 12% solution that included water and flavorings. However, although a general cleaning and sanitizing of the blades was performed daily, the equipment was completely disassembled for cleaning and sanitizing was performed only weekly. Any pathogens remaining in the solution reservoir or interior of needles after the general cleaning could contaminate product injected before the weekly sanitization. In order to address this source of contamination, industry developed guidelines (BIFSCO, 2005) on pathogen control during tenderization and injection.

Smoking or Partial Cooking:

Smoking, partial cooking and char-marking involve heating the product to a temperature that does not achieve an appreciable reduction of pathogens. The internal temperature of the product is below 110°F. The heat process is sufficient to create a char-mark on the product surface or set surface breading. The cold smoke is the application of smoke flavor without cooking or appreciably heating the product. The temperature range for cold smoking is in the range of 80-100°F but no higher than 120°F.

Char-marked patties are defined in the 1999 final rule as meat patties that have been marked by a heat source and that have been heat processed for less time or using lower internal temperatures than those temperatures (151°F and above) listed in 9 CFR 318.23(b)(1). In other words, the patties would not be heated to a time and temperature that would produce a fully-cooked product. In the 1993 rule (58 FR 41138), "Heat-Processing Procedures, Cooking Instructions, and Cooling, Handling and Storage Requirements for Uncured Meat Patties," the cooking or char-marking step could not result in an internal temperature of higher than 70°F. This requirement was removed on a subsequent rule on performance standards but illustrates that the internal temperature of a char-marked patty would not produce a RTE product. Char-marked or partially cooked patties also have to meet the regulatory requirements for cooling as discussed below under Cooling.

Heating or par-frying a breaded product sufficient only to set the breading will not produce an RTE product but it may appear fully-cooked to the consumer. Such confusion regarding appearance and whether the product is RTE have resulted in foodborne illnesses as discussed below in the Packaging/Labeling section.

In cold smoking or low temperature smoking, the smoke flavor is added to the product without producing a fully cooked product. A low temperature smoked product must be fully cooked by the consumer or food preparer before consumption. The advantage to the processor is less shrinkage. To adequately add the smoke flavor to poultry in a low temperature smoking process, the product is smoked for 18 hours at 100 to 120°F (Mast, 1978), whereas as fully cooked smoked poultry product would have to reach an internal temperature of at least 155°F.

Slow partial-cooking processes, such as those for bacon, involve low temperatures for long times. In 1999, FSIS published the final rule (64 FR 732) "Performance Standards for the Production of Certain Meat and Poultry Products" (USDA, 1999). Appendix A, "Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products," of that rule stated "Dwell times of greater than 6 hours in the 50°F to 130°F range should be viewed as especially hazardous, as this temperature range can foster substantial growth of many pathogens of concern." Especially troublesome would be the formation of staphylococcal enterotoxin since subsequent cooking by the consumer would not destroy it. Taormina and Bartholomew (2005) noted that bacon processing generally spans an 8 hour period from smoking and cooling to 45°F. They noted that while peak temperatures generally range from 122 to 126.5°F certain areas of the pork bellies can reach 131°F. Therefore, growth of bacterial pathogens, such as *Clostridium perfringens*, *Clostridium botulinum* and *Staphylococcus aureus*, must be addressed.

Burnham et al. (2006) developed a predictive tool for the safety of slow cooking of pork products and identifying critical limits. In their study, pork bellies pumped with a cure solution (25% (w/v) NaCl solution, 22% water, 11% (w/v) sugar, smoke flavor (4.5%), 1.75% sodium nitrite and other salts, and 0.25% proprietary ingredients) were slow cooked for 6 hours with additional times of 12 and 18 hours. Their results indicated that no meaningful growth of *S. aureus*, *Salmonella*, and *E. coli* O157:H7 occurred relative to time zero.

Cooling:

FSIS determined that product cooling is a critical control point in the production of a safe product. The 1999 final rule established a performance standard of no more than 1-$\log_{10}$ growth of *Clostridium perfringens* and no growth of *Clostridium botulinum* was established for meat patties. This limit on growth can also be applied to poultry patties. This growth limit was based the results on the National Baseline Surveys (USDA, 1996) which estimated the amount of *C. perfringens* in the meat and poultry products and the level of what would be the permissible level of *C. perfringens* in a finished product that would result in a foodborne illness. A detailed explanation is provided in the Technical Paper posted on the FSIS website at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/95-033F/95-033F_tech_paper.pdf. Appendix B, "Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization)," of the 1999 final rule provides a safe harbor for product cooling. Using the compliance guidelines enables the establishment to achieve the required limit on *C. perfringens* growth and no growth of *C. botulinum*. However, using the compliance guidelines for partially-cooked patties may not be practical since the initial temperature for fully-cooked patties is substantially higher than any temperature that a char-marked patty will attain. Nonetheless, an establishment must have supporting documentation that their rate of product cooling meets the stabilization performance standard.

A study by Taormina and Bartholomew (2005) examined the growth of *C. perfringens* and *Staphylococcus aureus* in bacon that was smoked and cooled for 15 hours. They noted that some processors use brine showering followed by blast chilling to cool the pork bellies but

other processors only use blast chilling. The maximum amount of time to cool the bellies to 45°F was ≤3 hours. Ground and whole pork bellies were inoculated with the two pathogens. The study demonstrated less than 1 log growth of *C. perfringens* occurred in both ground and whole bellies during the normal smoking and cooling conditions. Under normal cooling, *S. aureus* increased in ground bellies by 2.38 logs without smoke during but only increased by 0.68 logs when smoke was added. However, when cooling was extended to 15 hours, the growth of *S. aureus* in both whole and ground bellies increased by approximately 4 logs. At 15 hours, *C. perfringens* showed a <1 log increase in the smoked ground bellies but 3.93 log increase in the ground bellies. In contrast to the ground bellies, no growth of either pathogen was observed in whole bellies. The researchers concluded that cooling smoked whole belly bacon from 120 to 45°F in 15 hours did not present a food safety hazard from either *C. perfringens* or *S. aureus*. Taormin et al. (2003) previously had concluded that processed meat products cured with sodium nitrite are not at risk for *C. perfringens* growth.

The ARS Pathogen Modeling Program (ARS PMP) (available at: http://ars.usda.gov/Services/docs.htm?docid=6788) can be used as a tool in determining if the cooling rate is adequate but cannot be used by itself to verify that the performance standard was achieved. The ARS PMP does not always provide the most conservative evaluation of cooling, and may underestimate the amount of growth of *C. perfringens* occurring during cooling. For example, a study by Juneja and Thippareddi (2004) on the cooling of marinated ground turkey breast for 15, 18, and 21 hours showed a 3.83, 4.66, and 5.07 log growth of *C. perfringens*. Using the upper confidence limit (UCL) results of ARS PMP, the log growth of *C. perfringens* was 2.61, 4.30, and 5.84 for the 15, 18, and 21 hours used in the study. The average results, which many individuals using the program refer to, from the ARS PMP will be lower than the UCL results, and thus, underestimate growth even more. Since cure is the only factor that can be included in the ARS PMP and marinade ingredients cannot be entered into the model, any effect of marinade would not be reflected in the results from the ARS PMP. Rather than establishing an adequate cooling rate that would meet the performance standard, the ARS PMP would more helpful in evaluating cooling deviations.

Rework:

Establishments may rework product because the original process was inadequate, packaging problems, post-process contamination, etc. The problems range from safety to quality issues. Rework is product that is partially processed or finished product added back into the formulation at a rate of about 5 percent. If bacterial growth occurs before the rework is added back into the processing line, this would increases the bacterial load beyond that which the process is validated to eliminate. Bacterial growth can occur if product held for rework is maintained above 40°F for an extended period. Daskalov et al. (2006) assessed the effect of including contaminated rework in two cooked sausage formulations. The sausages containing inoculated emulsion, simulating contaminated rework, added to the product formulation showed a slightly greater number of surviving *L. monocytogenes* CFU/g after heating and after subsequent storage at 50°F than the sausages without inoculated emulsions. Even though the products in this category are not RTE, the

bacteria load may increase beyond the level intended to be addressed by the final cook, whether applied by a food preparer or a consumer.

Packaging/Labeling

Because heat-treated, not fully cooked, not shelf-stable meat and poultry products are not RTE but may appear so, it is important that the labeling alert the consumer or customer that the product is not RTE and provide instructions for handling the product to prevent a foodborne illness. The USDA regulations on safe food handling instructions (USDA, 1994) requires that products that are raw or those that have not undergone a process to render them RTE must have specific safe handling instructions. FSIS Directive 7235.1 provides guidelines on proper application of mandatory safe handling statements (FSIS, 1994). Since the products in this category are not shelf-stable, they will have to be maintained at or below the minimum temperatures for growth. These temperature ranges are listed in the Receiving Raw Meat and Poultry above.

A survey of food preparers in Oregon (Raab and Woodburn, 2001) indicated that while most (85% of the 100 surveyed) reported seeing the label only 30% reported changing practices because of the labeling and 26% usually read the label when cooking. The results of Behavioral Risk Factor Surveillance System (BRFSS) surveys in seven states indicated that 51% of 14,262 respondents reported seeing the label (Yang et al., 2000). Of the 51%, 79% remembered reading the label. Of the 79% reading the label, 37% reported changing their raw meat preparation methods. On the other hand, reported food handling practices in the Oregon study (Raab and Woodburn, 2001) reflected label recommendations to keep product frozen or refrigerated (99%), avoid cross contamination by hand washing (84%), and thorough cooking of hamburger (71%). However, these surveys only focused on raw meats and poultry, but not partially cooked products. The importance of labeling for partially cooked products is highlighted by the occurrence of Salmonellosis linked to the consumption of breaded poultry products.

In 2005, salmonellosis among consumers in Michigan and Minnesota was associated with the consumption of microwavable poultry entrees that were not RTE (NRTE) but appeared to be RTE. As a consequence, the consumers did not fully cook the entrees. *Salmonella* Typhimurium and *Salmonella* Heidelberg were identified in the course of epidemiological investigations (NACMCF, 2006). Another breaded product, frozen chicken nuggets and strips, was associated with foodborne illnesses in 2002. The frozen chicken nuggets and strips were determined to be contaminated with *Salmonella* Heidelberg (MacDougall et al., 2004). These illnesses from both 2002 and 2005, the products were par-fried to lend a cooked appearance although the meat was not fully cooked. MacDougall et al. (2004) identified the cooked appearance and inadequate labeling as contributing to consumer confusion in 2002. Additional cases of salmonellosis in Minnesota due to *Salmonella* Enteritidis have been attributed to stuffed chicken that appeared RTE but were in actuality NRTE (USDA, 2006).

A special problem is presented by whole muscle products that may be char-marked and blade tenderized or injected. During mechanical tenderization, the blades or needles can transfer microorganisms from the surface of the meat to the interior (Johnston et al., 1978; Gill and McGinnis, 2004; Gill et al., 2005; Sporing, 1999). Sporing (1999) reported that overall the blade tenderization process transferred 3 – 4 % of the surface microorganisms to the center of the muscle. If products that have been mechanically tenderized or injected are not adequately labeled, the consumer or other food preparer may not apply a full cook to the products. Although no foodborne illnesses have been linked to partially cooked and injected products, foodborne illnesses from *E. coli* O157:H7 have been linked to injected products (Laine et al., 2005). In addition to be marked as injected or mechanically tenderized, such products should also contain instructions for cooking.

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PRODUCT WITH SECONDARY INHIBITORS, NOT SHELF STABLE (03I)

Some of the products in this HACCP category, such as semi-dry fermented sausages, are similar to products in the heat treated, shelf stable and not heat treated, shelf stable categories except the finished products are not shelf stable but are ready-to-eat (RTE). Other products in this category, such as country cured ham, may be not RTE (NRTE). These products do not receive the amount of drying, or reduction in water activity, needed to make them shelf stable. Consequently, bacterial contamination after processing can result in growth of the contaminating pathogens, such as *Salmonella* or *Listeria monocytogenes*. In addition, the heating step in the process is below that normally associated with heat-treated products—120°F or above. Examples of perishable, not shelf stable, meat and poultry products with secondary inhibitors include semi-dry fermented sausages (e.g., cervalet, soft salami, and summer sausage) and country style or country cured ham.

The steps in the process are those cited in the FSIS Generic HACCP Model for Meat and Poultry Products, Not Shelf Stable. The steps listed below include both control points and critical control points in the process for microbiological food safety hazards only. The critical control points (CCPs) are those points or steps in the process at which control or action can be applied to eliminate, prevent, or reduce a food safety hazard to an acceptable level. The control points are those steps to control potential food safety hazards but which will not result in the elimination, prevention, or reduction of the food safety hazard. However, control points can reduce the hazard and critical limit that must applied at the critical control point. If growth or contamination is not controlled at these points, the level of pathogens may exceed the level of reduction needed and which the validated process is designed to achieve. The CCPs for cured products are salting, equalization, and drying/ripening, and for fermented products are fermentation and drying/ripening.

Receiving Raw Meat and Poultry

The temperature of the in-coming meat and poultry must be maintained below that allowing growth of pathogens. *Salmonella* is a pathogen of concern in raw meat products, and *Escherichia coli* O157:H7 represents a potential health hazard in beef products. *Salmonella* and *Campylobacter* are the primary pathogens of concern in poultry products. If the temperature of the product is not maintained at or below 40°F, these pathogens can growth. The optimum temperature growth ranges for *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 are 95-109.4°F, 107.6-109.4°F, and 95-104°F, respectively (Roberts et al., 1996). However, *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 can grow, albeit slowly, at temperatures of 41.4°F, 89.6°F, and 44.6-46.4°F, respectively. *E. coli* O157:H7 can grow rapidly at 50°F.

Receiving Non-meat/Non-poultry Food Ingredients

Non-meat and non-poultry ingredients include salt, sugar, spices, etc., which may contain pathogens and a high number of microorganisms per gram. Hara-Kudo et al. (2006) isolated two *Salmonella* spp. from black and red pepper (at least 1 CFU in 25 grams of

sample). The aerobic bacterial count, a general indicator of sanitation, of garum masala, tumeric, curry powder and paprika was greater than 5.39 CFU/g. Vij et al. (2006) reported an increased number of recalls of dried spices due to bacterial contamination. Paprika was the most frequently involved in the recalls. Of 12 recalls due to bacterial contamination, all but one was contaminated with *Salmonella*. These authors also noted that paprika contaminated with low numbers of *Salmonella* was the cause of a nationwide outbreak. *Bacillus cereus*, which is a pathogen of concern during product cooling, is a common contaminant of spices (McKee, 1995).

Storage (Frozen/Refrigerated) Raw Meat and Poultry

The same reasoning for receiving raw meat and poultry applies to storage of these products. As noted above, temperatures above 40°F will permit slow growth of pathogens. The minimal growth temperatures for *Salmonella* and *E. coli* O157:H7 are only slightly above 40°F.

Processing – Cured Products

For cured products, such as country cured, not shelf stable ham, the CCPs are cure contact time, equalization, and drying/ripening. The lethality of the process for *Salmonella* and other pathogens achieved in a salt-cured product will depend on the interaction of salt content, pH, time and temperature of curing, cold smoking/drying and aging. These steps are necessary to prevent, eliminate, or reduce to an acceptable level the pathogens of concern- *Salmonella*, *Trichinella spiralis*, and *Listeria monocytogenes*. This combination of steps represents hurdles to bacterial growth since each step alone would not suffice to meet the pathogen reduction requirements in an establishment's HACCP plan. The regulatory requirements in 9 CFR 318.10 for the elimination of trichinae from pork products may not eliminate the bacterial pathogens. The establishment's HACCP plan must address the bacterial pathogens of concern.

Cure Contact Time (Salting)

During a dry salting, the ham is covered with a salt and cure mixture and held at 40°F for at least 28 days or no less than 1.5 days per pound of ham (9 CFR 318.10). The time for the salting phase for shelf stable country cured hams is longer than it is for non-shelf stable hams. The salting rapidly reduces the amount of water available for bacterial growth (i.e., decreases the water activity, a_w) (Reynolds et al., 2001) and the hold temperature inhibits bacterial growth (Leistner and Gould, 2002). If brine (salt in a water phase) is used instead of a dry salt-cure rub, it is usually ranges from 60% to 70% of saturation (0.87 to 0.82 a_w) (Huang and Nip, 2001). A water activity below 0.93 will prevent the growth of most pathogens except *Staphylococcus aureus* (Farkas, 1997). Portocarrero et al. (2002a) concluded from their results that the higher salt content and lower a_w values on country cured ham are important in controlling the growth of *S. aureus* and enterotoxin production.

Equalization (Post-salting). The equalization phase is the time after the minimal cure contact time, removal of the excess salt, and before placement in the drying room. During

the equalization period, the salt permeates to the inner tissues of the pork muscle. The concentration of salt with resulting decrease in water activity will inhibit the growth of bacteria during ripening (Leistner and Gould, 2002).

Drying/Ripening

From the work of Reynolds et al. (2001), it appears that most of the lethality for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* occurs in this step. However, since the ham products in this HACCP category are not shelf stable, the growth of *L. monocytogenes* may not be inhibited if the water activity is above 0.92, if the product is contaminated in the post-lethality environment. Portocarrero et al. (2002b) judged that a longer drying/ripening time to attain a lower a_w, such as that found with shelf stable country cured hams, is needed to eliminate *L. monocytogenes*. They demonstrated that a cold smoke, smoking at a low temperature, was not sufficient to eliminate *L. monocytogenes* under their processing conditions but did provide a >6 $\log_{10}$ reduction of *L. monocytogenes*. In addition, the Portocarrero study (2002b) found that the level of *E. coli* O157:H7, which would not be expected in a ham, decreased faster than *Salmonella* or *L. monocytogenes*. They concluded that *Salmonella* and *E. coli* O157:H7 do not represent a potential health hazard in properly prepared country cured hams, but that *L. monocytogenes* does represent a potential problem. Reynolds et al. (2001) demonstrated a 5.0 $\log_{10}$ reduction of *Salmonella* and *E. coli* and that the proliferation of *S. aureus*, and hence enterotoxin production, was not a concern. However, the country cured hams in these studies were shelf stable products. A ham that is not shelf stable will have to be refrigerated to prevent the growth of pathogens.

Processing – Fermented Products

For fermented products, such as a soft salami, the CCPs are fermentation and drying/ripening. Four pathogens associated with fermented sausage products are *S. aureus*, *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7. For *S. aureus*, the release of a heat stable enterotoxin after it has achieved a density of at least 10^5 CFU/g rather than the bacterium itself is responsible for foodborne illness. *E. coli* O157:H7 is a pathogen of concern in those products containing any amount of beef.

Fermentation and Drying/Ripening

Fermentation and drying/ripening are 2 distinct steps in the process. The discussion of both is combined for clarity.

Growth of *S. aureus* is inhibited by the competitive growth of lactic acid bacteria, such as lactobacilli and pediococci (Hayman, 1982; Tatini, 1973). Large amounts of acid produced during longer fermentation should inhibit or reduce any *S. aureus*. In one study (Smith and Palumbo, 1978), a >6 $\log_{10}$ reduction of *S. aureus* was attributed to production of lactic acid. However, inadequate fermentation procedures, temperature abuse during fermentation, or an initial large number of *S. aureus*, as has occurred when contaminated starter culture is used, may result in the growth of *S. aureus* and the release of enterotoxin.

The main microbial hazard associated with this fermentation step is *S. aureus* proliferation and the elaboration of its enterotoxins. The degree-hours concept is the control measure used for this biological hazard (American Meat Institute Foundation, 1997). Many establishments identify this control measure as a CCP in the HACCP plan. However, some establishments may address the degree-hours concept in a prerequisite program instead of as a CCP in the HACCP plan. In addition, there have been cases, where some establishments have *not* addressed the degree-hours concept at all in their HACCP system. In these cases, there is a significant public health concern.

Simply put, the degree-hours concept is the time, in hours, for the product to reach a pH ≤ 5.3 multiplied by the number of degrees the fermentation chamber is over 60°F (minimum growth temperature for *S. aureus*). The degree-hours is calculated for each temperature used during fermentation, but a constant chamber temperature may be used. The number of degree-hours is limited by the highest temperature in the fermentation process prior to reaching a pH of 5.3 or less. For example, if the highest chamber temperature is less than 90°F, the process is limited to fewer than 1200 degree-hours; fewer than 1000 degree-hours if the chamber temperature is between 90 and 100°F; or fewer than 900 degree-hours if the chamber temperature is greater than 100°F (American Meat Institute Foundation, 1997).

Both *Salmonella* and *E. coli* O157:H7 have been isolated from fermented sausage products. The great variety of products and processing procedures hinder determining if an x-$\log_{10}$ reduction of one pathogen will always produce a y-$\log_{10}$ reduction of the other. This point is illustrated by two studies on the reduction of *Salmonella*, one in Lebanon bologna and the other in pepperoni. In a Lebanon bologna process (Smith et al., 1975b), a 3 to 4 $\log_{10}$ reduction of *Salmonella dublin* and a reduction of *Salmonella typhimurium* to undetectable levels was observed by the end of fermentation if starter culture was used. Little reduction in the numbers of salmonellae was observed if aged beef without starter culture was used. Similarly, Bacus (1997) noted that contamination of fermented meat products with *Salmonella* most likely results from an inadequate lactic acid production or a highly contaminated raw product. In addition, the Lebanon bologna study demonstrated the effect of different processes, with and without starter culture, on the reduction of *Salmonella* and the difference in reduction between two serotypes of the same organism. In a pepperoni process (Smith et al., 1975a), *Salmonella dublin* was detected after fermentation and subsequent 43 days of drying but *Salmonella typhimurium* was undetectable after 29 days of drying. The reduction of *S. dublin* and *typhimurium* occurred at different stages in the process for the Lebanon bologna and pepperoni products, and *S. dublin* appeared more resistant to both fermentation and drying than S. *typhimurium* in both products.

Various studies have shown that fermentation and drying resulted in about a 2 $\log_{10}$ reduction of *E. coli* O157:H7 (Ellajosyula et al., 1998; Faith et al., 1997, Glass et al., 1992). Glass et al. (1992) reported that *E. coli* O157:H7 decreased by about 2 $\log_{10}$ CFU/g after fermentation, drying, and storage at 4°C for 6 weeks following the end of 18-21 day drying cycle for a fermented sausage formulation. However, a 5 to 6 $\log_{10}$ reduction of *E. coli* O157:H7 was observed in pepperoni sticks following fermentation, drying, and 2 weeks of storage at ambient temperature (21°C) (Faith et al., 1997). In one of the few studies that

compared the combined effect of fermentation and drying on both *Salmonella* and *E. coli* O157:H7, Ellajosyula et al. (1998) observed that the reduction of *Salmonella* and *E. coli* O157:H7 in Lebanon bologna was less than 2 $\log_{10}$ after fermentation to pH 4.7. In this study, *Salmonella* was equally or significantly ($p<0.01$) less resistant than *E. coli* O157:H7 to various combinations of pH levels achieved after fermentation and subsequent heating at 110°F to 120°F. Fermentation to pH 5.2 or 4.7 followed by heating at 110°F to 120°F for specified times (e.g., 110°F for 20 hours or 120°F for 3 hours) resulted in >7 $\log_{10}$ reduction of both *Salmonella* and *E. coli* O157:H7. This study shows that a final heating step may be necessary to achieve the proposed $\log_{10}$ reduction of both *Salmonella* and *E. coli* O157:H7 in fermented sausage products.

The Blue Ribbon Task Force (Nickelson II et al., 1996) listed 5 options for achieving a 5D or equivalent inactivation of *E. coli* O157:H7. The listed options were: 1) utilize a heat process as listed in Appendix A to the final rule "Performance Standards for the Production of Certain Meat and Poultry Products;" 2) include a validated 5D inactivation treatment; 3) conduct a "hold and test" program for finished product; 4) propose other approaches to assure at least a 5D inactivation; and 5) initiate a HACCP system that includes testing of raw batter and achieving at least a 2-$\log_{10}$ reduction of *E. coli* O157:H7. Option 1 refers to compliance guidelines used by industry for applying a heat treatment to achieve a 6.5 $\log_{10}$ reduction of *Salmonella,* which may be too severe for some products. Options 3 and 5 involve testing of the finished product or ingredients, and are, therefore, dependent on the rigor of the testing program. Option 4 is an opportunity for industry or academia to validate processes that achieve a 5 log reduction of *E. coli* O157:H7. Option 2 was the intent of the Task Force research. The results from the Task Force studies indicated fermentation temperature, product diameter (55 or 105 mm), and product pH were determining factors in achieving a 5 $\log_{10}$ reduction of *E. coli* O157:H7. For example, at a pH ≥5.0 and an incubation temperature of 70°F, a heat treatment is needed regardless of product diameter. On the other hand, if the incubation temperature is 110°F, holding the product at incubation temperature would achieve at least a 5 $\log_{10}$ reduction of *E. coli* O157:H7 without an additional heat treatment for all diameter products and pH levels - except 55 mm sausage with a pH ≥5.0. (Note: the reduction is based on the average reduction achieved in the study minus 2 standard deviations.)

Acid adaptation and acid tolerance to the lowered pH in fermented products also contribute to pathogen survival and must be considered when validating processes for fermented meat and poultry products. Acid tolerance and adaptation have been observed in both *Salmonella* and *E. coli* O157:H7. Tsai and Ingham (1997) reported that acid adaptation enhanced the survival of both *Salmonella* and *E. coli* O157:H7.

While some researchers observed only a 1 $\log_{10}$ decrease of *L. monocytogenes* during fermentation and drying (Johnson et al., 1988), others (Glass and Doyle, 1989) have observed a >4 $\log_{10}$ reduction. *L. monocytogenes* has been detected in fermented sausage products before and after processing (Farber et al., 1988). It is the most frequently isolated pathogen in the FSIS monitoring program for fermented sausages. However, it is not known whether isolation of *L. monocytogenes* in the FSIS fermented sausage monitoring program resulted from environmental contamination, an inadequate process, or both.

Despite its prevalence in fermented sausage products, no foodborne illnesses have been linked to *L. monocytogenes* in fermented sausages and only rarely for meat products in general. *L. monocytogenes* is not a reference organism for fermented sausages. However, the finding of *L. monocytogenes* in the finished product would result in regulatory action as provided for in the Agency's fermented sausage monitoring program.

Rework

Rework is product that is partially processed or finished product added back into the formulation at a rate of about 5 percent. The possibility exists that reworked product becomes contaminated from a food contact surface or bacterial growth occurs before the reworked product is added back into the formulation. For example, product could be exposed to a food contact surface contaminated with *L. monocytogenes* in the post processing environment. If bacterial growth occurs before the rework is added back into the processing line, this could increase the bacterial load beyond that which the process is validated to eliminate. Bacterial growth can occur if product held for rework is maintained above 40°F for an extended period. Daskalov et al. (2006) assessed the effect of including contaminated rework in two cooked sausage formulations. The sausages containing inoculated emulsion, simulating contaminated rework, added to the product formulation showed a slightly greater number of surviving *L. monocytogenes* CFU/g after heating and after subsequent storage at 50°F than the sausages without inoculated emulsions.

Labeling and Packaging

As for any RTE product exposed to the post-lethality processing environment, a major public health concern is contamination of the product by *Listeria monocytogenes*. An establishment can address *L. monocytogenes* in the processing environment by any of the three Alternatives described in the final rule (68 FR 34207), "Control of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products."

Since the products in this category are not shelf stable, they cannot use the water activity of the product as an antimicrobial agent under the Alternative 2b. However, other antimicrobial agents described in the "Compliance Guidelines to Control *Listeria Monocytogenes* in Post-Lethality Exposed Ready-to-Eat Meat and Poultry Products" can be used to control *L. monocytogenes*. The compliance guidelines provide guidance for any of the three Alternatives in the final rule. The compliance guidelines are available on the FSIS web site at: http://www.fsis.usda.gov/oppde/rdad/FRPubs/97-013F/LM_Rule_Compliance_Guidelines_May_2006.pdf.

Some chorizos, soujouk, and other typically NRTE sausages are fully processed and made RTE. Thus, proper labeling is crucial for consumer protection. More specifically the product package should include the following conspicuous labeling features: Safe handling instructions, if product is not processed or marketed as a RTE product; terminology indicating that the product must be cooked for safety (e.g., Raw, Uncooked, or Cooked thoroughly), if it is not obvious that the product is raw; cooking and preparation instructions validated to ensure food safety; and the nutrition facts, if present, should

include a serving size based on the ready to cook reference amount (see Resource 1 of FSIS Directive 10, 240.4).

Since the products in this category are not shelf stable, they will have to be maintained at or below the minimum temperatures for growth. These temperature ranges are listed in the "Receiving Raw Meat and Poultry" section of this literature review.

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PORK SLAUGHTER (03J)

Swine slaughter is an open process with many opportunities for the contamination of the pork carcass with potentially pathogenic bacteria. However, it does not contain any point where hazards are completely eliminated. Data on the prevalence of various pathogenic bacteria*, Listeria monocytogenes Salmonella spp. Campylobacter jejuni and C. coli, Staphylococcus aureus, Yersinia enterocolitic* and *Aeromonas hydrophila* in pigs, their growth and survival characteristics, and ability to become established on the slaughter line are presented. This literature review covers the processing steps from lairage through chilling and packaging. The major contamination points during swine slaughter are pig-related, such as fecal and pharyngeal, and environmental. Hazard Analysis Critical Control Point (HACCP) and sanitation practices in swine slaughter must be focused on limiting this spread. The pathogenic bacteria show differences in their general mechanism of distribution. The major contamination source of *Campylobacter* spp., *Salmonella* spp. and Y. *enterocolitica* is the pig, and the contamination of carcasses with these bacteria may be limited, provided that only strict slaughtering procedures are used. Other organisms, such as *Aeromonas* spp., *Listeria monocytogenes*, and S. *aureus*, can be endemic in the processing environment. Since endemic bacteria can be controlled by proper cleaning and disinfection, these organisms are useful as indicators for the success of sanitation.

Specific areas in the pork slaughter process addressed in the literature review describe specific considerations for food safety hazards at each of the following points in the slaughter process: lairage, live receiving/pen holding; stunning/sticking/bleeding; scalding; dehairing; gamberling/singeing; polishing/shaving; pre-evisceration wash; hoof trimming; head dropping/removal; bunging; carcass splitting/evisceration; final trim/final wash; chilling/cold storage; and shipping.

Live Receiving/Pen Holding

It has been reported that pork carcass contamination with *Salmonella enterica* is primarily related to intestinal *S. enterica* infections (Craven, 1982; Morgan, 1987; Widders, 1996). It is assumed that the more *S. enterica* that is carried into the slaughter process, via the pig's intestines, the greater the risk of equipment and final product contamination. Therefore, reductions in pre-slaughter infection rates should result in increased pork safety. A number of studies have reported that *S. enterica* isolation rates in market swine are 3 to 10 times higher after transport and slaughter compared to rates measured on the farm (Berends, 1996; Hurd, 2001; Shots, 1962; Williams, 1967, 1970). One possibility for this increase in isolation rates is long-term lairage (greater than 12 h) in contaminated abattoir holding pens (Craven, 1982; Hansen, 1964; Kampelmacher, 1963; McDounagh, 1958; Morgan 1987). In the United States, most abattoirs report that they try to avoid holding pigs for more than 6 to 8 h. However, a 2-h holding period is recommended to improve meat quality (Berg, 1998; Grandin, 1994; Warriss, 1992). The stress of transport has also been suggested as a reason for increased *S. enterica* shedding (T. J. Stabel and P. J. Fedorka-Cray, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., p. 60, 1999). The physiological changes associated with stress might encourage the recrudescence of latent carriers or it might increase the

susceptibility of noncarriers to new infection. Immunological parameters, such as cortisol or beta-endorphins, are increased after transport (Stabel and Fedorka- Cray, Abstr. 99th Gen. Meet. Am. Soc. Microbiol.).

However, few studies have demonstrated a direct increase in *S. enterica* shedding or infection due to these physiological changes. Williams and Newell described increased shedding after transport. However, this study used a small number of pigs (n <20) and the differences in isolation rates were not statistically significant. Isaacson et al. (1999) reported increased isolation rates after transport but only if the pigs did not fast before transport. They concluded that transport stress alone did not contribute to the increase in isolation rates. In support of this conclusion, no difference was demonstrated between directly shipped pigs and those stressed by mixing, fasting, and 18 h of holding in a clean, disinfected facility.. A weakness in that study, and in others, is the before and after comparison of unmatched sample types and amounts (Hurd 2001, Williams 1970). For example, by using 1 g of feces, *S. enterica* was recovered from 3.4% of pigs tested on the farm. However, after transport and holding and by using colon contents (10 g), cecal contents (10 ml), and ileocecal lymph (ICL) nodes, 71.8% of the same pigs (196 of 273) were positive. Increasing the volume of feces from 1 to 10 g has been shown to double the sensitivity (Berends et al., 1996). The inclusion of multiple samples from the same pig will increase the likelihood of detecting a positive pig, and the culture of ICL nodes may detect latent nonshedders. Therefore, unmatched comparisons may be invalid. Additionally, many studies suffer from the possibility of in-plant sample contamination. Samples are often collected from viscera sets after frequent handling along the conveyor belt. It is possible that some isolates were from workers or from the environment and not from the pigs. The objective of this study was to compare, by using identical sample types, the *Salmonella enterica* prevalences and serovar diversities between pigs necropsied on the farm and those necropsied at the abattoir after transport and holding. A more recent study by Hurd (2002) necropsied 567 market weight pigs (>70 kg) from six herds. Pigs were alternately assigned to be necropsied on the farm or at the abattoir. One-half of the group was sent in clean, disinfected trailers to slaughter at a commercial abattoir. After transport (mean distance, 169 km) and 2 to 3 h of holding in antemortem pens, these pigs were necropsied. The 50 pigs remaining on the farm were necropsied the following day. The same sample types and amounts were collected for *S. enterica* culture at both locations. Results showed a sevenfold-higher ($p < 0.001$) *S. enterica* isolation rate from pigs necropsied at the abattoir (39.9%; 114 of 286) than from those necropsied on the farm (5.3%; 15 of 281). This difference was also observed for each individual herd. All sample types showed a significantly higher prevalence when comparing abattoir to on-farm collection, respectively: lymph nodes, 9.15 versus 3.6%; cecal contents, 13.6 versus 1.8%; 1 g of fecal matter, 25.2 versus 0.7%. Recovery of additional serovars at the abattoir suggests the pigs are receiving *S. enterica* from extra-farm sources. This study demonstrates that rapid infection during transport, and particularly during holding, is a major reason for increased *S. enterica* prevalence in swine. This finding identifies the holding pen as an important *S. enterica* control point in the pork production chain. Sanitation of the holding pen, minimizing cross contamination between herds in the holding pen, and minimizing the amount of time pigs are held in the holding pen should be emphasized to minimize *Salmonella* contamination of hogs entering the slaughter facility.

During lairage, pathogenic bacteria may spread from infected to non-infected pigs, as shown for *Yersinia* by Fukushima et al. (1990). Herds should be handled separately, if possible, and cleaning and disinfection should be performed between herds if slaughter operations allow.

The time of the last feed before slaughter will affect the fullness of the stomach; a full stomach may pose a higher risk of puncturing during the dressing.

Stunning/Sticking/Bleeding

Spoilage deep within the tissues derived from healthy animals is initiated by contamination at the sticking process (Jensen, 1954).

Scalding

During scalding, a reduction in the bacterial levels takes place, but the extent of reduction for a specific bacterial species depends on the heat resistance of the bacterium (Bergdoll, 1989; Stern and Kazmi, 1989; Sijrqvist and Danielsson-Tham, 1990; Nishikawa et al., 1993; Siirquist, 1994) and the time/temperature combinations used. During normal scalding procedures (6 min at 60°C) a log-reduction of several times of *Aeromonas* spp., *Campylobacter* spp., *L. monocytogenes, S. aureus* and Y. *enterocolitica* is achieved. In contrast, for some *Salmonella* spp. the reported heat resistance is somewhat higher than for the other pathogens listed (D'Aoust, 1989).Scalding at a relatively high temperature for a short period in an alkaline environment has the greatest effect in decreasing the surface microbial levels. This is in agreement with other time temperature studies which support a minimum scalding temperature of 60°C. Pelczar and Reid (1965) demonstrated a reduction in microbial load to 0.3% of the pre-slaughter levels at 60°C, but reduced killing of 1.7% reduction of pre-slaughter load with 58.5°C, and 12.5% at 54°C.

Scalding can be carried out on pigs either hanging or in vats using steam or recirculating water. The scalding procedure takes 6-8 minutes and the water temperature is 60-61.5°C. The reduction in bacterial numbers on the carcass during scalding depends on the time-temperature conditions used. The penetration by bacteria into the sticking wound during scalding is insignificant (Siirquist, 1990). The significance of filling the lungs with scalding water that becomes contaminated during the passage through the mouth and pharynx needs further elucidation. Pathogenic bacteria will then spread to the carcass and pluck set when the pluck set is removed, and possibly also to the pluck set during meat inspection that includes palpation and incision.

In one plant examined, carcasses were transported through the scalding tank by a platform rotating over the carcasses. The capacity of the dehairing machine limited the rate of movement through the scalding tank. Consequently the platform was halted in the ascending position each time the dehairing machine was full, thereby limiting the amount of contact time the backs of the pigs had with the heat. Increased microbial loads in the back area of pigs in this plant lead to discovery of this malfunction.

Dehairing

Dehairing machines consist of rotating drums equipped with scraper blocks that rotate the carcasses to remove the hairs. The skins of scalded pig carcasses are essentially free of both enteric pathogens and spoilage pathogens (Gill et al., 1995). Recontamination of the carcasses with these pathogens often occurs at dehairing. The detritus found in the area of the dehairing machine has long proven to be a source of cross contamination in the slaughter process, because feces are often voided from pork carcasses during this process. Pearce et al (2004) found an increase in *Salmonella* positive carcasses from 1%-7% after dehairing and a 2 $\log_{10}$ increase in mesophilic bacteria and coliforms. Rivas et al. (2000) found that bacterial counts in the dehairing equipment ranged from 4.4 $\log_{10}$ to 6.2 $\log_{10}$ cfu cm^{-2} 3h after slaughter had commenced. Pearce et al. (2006) recovered *S. typhimurium* from air samples at the dehairing equipment area assuming aerosolization of *S. typhimurium* due to contaminated carcasses or from the dehairing equipment itself. Gill and Bryant (1993) found detritus from dehairing machines at two different abattoirs contained large numbers of *Escherischia coli* and *Campylobacter,* up to 10^5 and 10^6 cfu/g, respectively, and *Salmonella* spp. being isolated in 50% of samples in quantities up to 10^5 cfu/g.

Dehairing equipment also has the potential to be a possible source of carcass contamination with spoilage bacteria. Gill and Bryant (1993) reported high numbers of acinetobacteria and pseudomonads in accumulated detritus and circulating waters of the dehairing equipment. Gill and Jones (1995) found high numbers (6/6 samples positive) of *Aeromonas* in the dehairing equipment detritus where the water temperature was 47°C as opposed to 57 °C where lower numbers of spoilage bacteria were isolated. Although scalding has been shown to reliably reduce pork skin microflora to gram positive types, studies conducted by Gill and Bryant (1992) documented gram negative flora post scald that they attributed to wash water thrown from the dehairing equipment by the revolving flails in two plants, and from general detritus in the dehairing area in another plant. Gill and Bryant (1993) found that washing after dehairing and raising the temperature of the circulating water in the dehairing machine could decrease the amounts of *Campylobacter, Salmonella, E. coli* and other spoilage bacteria on carcasses.

Gamberling/Singeing

Flaming and singeing are performed at 800-900°C and l,000°C, respectively, for l0–15 s. Singeing differs from flaming in the sense that the oven itself contributes to the heating of the carcass. Singeing for 10 s raises the surface temperature of the carcass to approximately 100°C. The high temperature used reduces the total count on the rind (Nerbrink and Borch, 1989), but is dependent on the method used, i.e., singeing or flaming, and the time. Gill and Bryant (1993) found a 2 log reduction in the numbers of *E. coli* during singeing. Thus, singeing/flaming is not sufficient in eliminating the bacterial contamination on the carcass surface, but it has a significant effect in reducing the contamination.

Singeing of pig carcasses has been reported to substantially reduce the numbers, and significantly alter the composition of the microflora on the skin. In such circumstances the

microbial load is reported to be augmented after the polishing operation (Gerats et al., 1981; Nerbrink and Borch, 1989). In contrast, other workers have observed little or no effect of singeing on the microflora, with numbers being unaffected or diminished after the polishing operation (Dockerty et al., 1970; Nickels et al., 1976). In a study by Gill and Bryant (in the International Journal of Food Microbiology, 1992), singeing apparently reduced flora numbers at one plant, but had no obvious effect at a similar plant while polishing reduced the flora numbers at both those plants. In contrast, at the smaller plant, in the study neither the combined dehairing-singeing-partial polishing operation or the final polishing altered the numbers of the flora. Although those various findings seem contradictory, they probably reflect common effects. It can be suggested that singeing reduces the flora over localized areas of a carcass, but reductions will be detected only if the area chosen for sampling was effectively heated during singeing. The locations of such areas are likely to be relatively constant on the carcasses leaving each singeing system, but those locations are unlikely to be the same for all systems. Thus, sampling from equivalent sites on carcasses leaving different singeing systems could yield the different results obtained at the plants. The common findings after polishing at both plants, of flora reduced in numbers but unaltered in compositions, would then be a result of the polishing redistributing the remaining flora evenly over each carcass surface. However, at the smaller plant, the carcasses were continuously recontaminated by the dehairing equipment in which they are rotated during the singeing and early polishing operations. Under such circumstances, the flora would remain invariant despite the singeing and polishing of carcasses.

Polishing /Shaving

Polishing and shaving can be sources of cross contamination on pork carcasses. During polishing, carcasses can become contaminated from the actual polishing equipment or due to redistribution of the flora remaining on the carcass after singeing. Polishing is performed by stainless steel scrapes or nylon brushes. The polishing contributes to the spread of bacteria surviving the singeing. Furthermore, the equipment is difficult to clean and sterilize, and bacteria may become established on the surfaces of the brushes and scrapes. In a study by Gill and Bryant (1993), *Campylobacter* spp. were retrieved on the rind after singeing and polishing at levels up to 6 cfu/cm^2. Numbers of bacteria on pig carcasses may increase during polishing (Nerbrink and Borch, 1989). Numbers of mesophilic bacteria and *Pseudomonas* spp. were, after flaming, 3.0 and <0.4 log cfu/cm^2, respectively, and, after polishing, 3.8 and 1.0 log cfu/cm^2, respectively.

While the entire carcass is shaved, the process in not uniform, concentrating on the removal of hair from those parts of the carcass least affected by the dehairing machine (the hind and fore shanks and the head). Sanitation of shaving equipment is necessary to avoid carcass to carcass transfer of contamination.

Pre-evisceration wash (antimicrobial)

Gill et al. (1995) found that the treatment with hot water of 85 degrees C for 20 seconds reduced the total numbers of bacteria two orders of magnitude, while non-thermoduric, spoilage bacteria were reduced from about 50% to about 10% of the population.

Head Dropping/Removal

Meat inspection procedures concerning the head represent a particular cross-contamination risk especially for *Salmonella spp*. and *Yersinia enterocolitica* (Borch, 1996). Pathogenic bacteria may be transported from the tonsillary region to other parts of the carcass by the knives and hands of the meat inspection personnel (Nesbakken, 1988). During the dressing of the head, further contamination may occur. The removal of the tonsils is carried out together with removal of the tongue, but even after careful tonsil removal, pieces of the surrounding pharyngeal tissue often remain on the head. Cutting and removal of head-meat in pigs should be carried out on a separate work table, in a separate room. This room should therefore be considered as an unclean area. Knives and equipment must not be used for cutting and deboning other parts of the carcass, and the flow of personnel into this room must be restricted. Knives, cutters and other tools/equipment used are likely to become contaminated by pathogenic bacteria that will subsequently be transferred to the carcasses.

Bunging

The rectum may be circumcised manually, or mechanically by means of a 'Bung cutter' which consists of a probe and a sharp rotating cylinder. The technique used during the dressing procedure will determine the extent of contamination of the carcass with fecal matter. In many countries, it is common to use plastic bags to seal off the rectum after loosening the circumanal skin. A process procedure which prevents the dissemination of any pathogenic bacteria present in feces to the carcass and subsequently to the cut meat is of great significance for the hygienic production of pork. Nesbakken et al. (1994) found that the use of the plastic bag reduced the incidence of Y. *enterocolitica* 0:3/biovar 4. Without the use of plastic bags, 10% of the carcasses were contaminated with the bacterium, as opposed to where plastic bags were used where 0.8% were contaminated. Furthermore, the single carcass (1 out of 120) found to be contaminated occurred at the exposed split surface, a contamination that is not likely to directly originate from feces.

Recommendations from these authors suggested that by incorporating the plastic bag technique into the slaughtering procedures, the meat industry would contribute to preventing the dissemination of *Y. enterocolitica* and other pathogens which spread via feces. Other technical solutions have also been tested. For example, by inserting a pre-frozen stainless steel plug into the anus prior to rectum-loosening and gut removal, a very tight seal is achieved minimizing the risk of fecal contamination to the carcass.

Carcass Splitting/Evisceration

Splitting of carcasses is done with automatic splitting machines. There is a risk that the splitter/saw will come into contact with the rectal incision or the head. The machines should be disinfected between each carcass. In some countries machines with automatic disinfection are used. If the machines are properly maintained and the line-speed does not exceed the capacity of the machines, reducing the time available for disinfection, the splitting process should not contribute substantially to carcass contamination.

Evisceration is considered to be one of the most important critical control points in the slaughter process. There is disagreement in the literature as to how much contamination occurs in pork slaughter due to the evisceration process, and this is likely due to variations in processes between plants. Borsch et al. (1996) consider evisceration to be a critical control point while others suggest that the low incidence of gut rupture, and lack of corrective action when it does occur, mean evisceration is better controlled using SOP and GMP (Bolton et al., 2002). Bolton et al. (2002) reported a decrease in the incidence of *Salmonella* during the evisceration process, when a single well trained employee performed the evisceration process, carcass inspection and trimming, at his own pace. By using one employee, the operations were performed properly, a two knife system was used (one knife is sanitized at 85°C while the other is in use).and there was no increase in the levels of bacteria on the carcasses. Berends et al. (1997) compared the process of routine evisceration to an "extra careful evisceration" process where knives and hands of workers were cleaned and disinfected after each manipulation showing that the hygienic practices work. Other studies done by Oosterom and Notermans (1983) and Childers et al. (1973) estimated that current routine evisceration processes contribute between 55-90% to the total number of *Salmonella* positive carcasses. In summary, when the intestines are removed, there is a risk of making holes in the intestinal tract so that fecal matter containing potentially pathogenic bacteria are spread over the carcass.

Normally the stomach is removed with the intestinal tract and it is important to cut the esophagus at the right distance from the stomach so that the stomach contents do not leak and contaminate carcass, liver and diaphragm, since stomach ingesta also contains *Salmonella* organisms. The training of operators is fundamental in order to prevent problems in these evisceration stages. If visible contamination occurs, this may be cut away, resulting in a reduction of microbial contamination, but will not result in a complete elimination of pathogens.

During traditional removal of the pluck set (kidneys, diaphragm, heart, lungs, esophagus, trachea, tongue with tonsils), the tongue and tonsils are removed along with the pluck set and hang together on a hook/conveyor. The spread of pathogenic bacteria from the tonsils and the pharynx to the carcass and the pluck set is unavoidable, thereby requiring a separate line for inspection of the pluck. Pathogenic bacteria such as *Yersinia* spp. and *Salmonella* spp. are present in high numbers on tonsils. The incidence of *Yersinia* spp. on tonsils, carcass fore-end and liver/diaphragm in a Danish study was found to be 72%, 14% and 17% (Christensen and Liithje, 1994).

Final Trim/Final Wash

Decontamination techniques for carcasses are targeted at reducing or eliminating bacteria that may be human pathogens as well as those that may cause meat spoilage. Generally conditions created by decontamination methods that lead to the reduction of overall levels of bacteria as measured by total aerobic plate count or total coliforms, provide some indication of the potential effects on pathogens. However, since this does not hold true in all cases, validation studies conducted in laboratory settings have specifically measured reductions of artificially inoculated bacterial pathogens (Huffman, 2002). Different methods of heat treatment of surface layers were suggested and evaluated. They involved hot water, steam and hot air and were tested on different carcasses. Steam has been shown to be effective in reducing the number of microorganisms on meat surfaces (James et al., 1998; Morgan et al., 1996). Gill and Bryant (1997) found that vacuum–hot water cleaning (water and steam temperature >82°C), pasteurizing treatments (105°C for 6.5 s) and subsequent spray-cooling of cattle carcasses can be operated in commercial practice to reduce log mean numbers of coliforms and *Escherichia coli* by >2 and log mean numbers of total aerobic bacteria by >1. Castelo, Kang, Siragusa, Koohmaraie, and Berry (2001) evaluated different treatments on pork trim. They used different combinations of water (cold and hot 82.5°C), hot air (510°C) and lactic acid. On both surfaces, lean pork trim tissue and fat-covered trim tissue the lower microbial populations were observed at samples treated by water and lactic acid. Treatment of pork trim did affect color of the meat. Pork mince prepared from trim treated with any of the treatment processes had lower initial microbial populations compared to the untreated samples. The water plus lactic-acid treatment provided the greatest microbial reduction and inhibition without large negative effects on quality attributes of the pork mince (Castelo et al., 2001a). Even though decontamination of meat may reduce the number of pathogens, higher growth of pathogens may occur during storage due to removal of competing non-pathogenic bacteria. Nissen et al. (2001) investigated the effect of meat decontamination (steaming and spraying with 0.2 M lactic acid) on growth and survival of pathogens in meats. Both decontaminated and untreated samples of pork were inoculated with *Salmonella enteritidis*, *Yersinia enterocolitica* and *E. coli* O157:H7, respectively, and stored at 10°C. For pork, no significant differences between decontaminated and untreated samples were observed.

Organic acids reduce bacterial counts on the meat surface layer; lactic acid is often used, as it is a natural meat compound produced during the postmortem glycolysis. Moreover, the lactate anion retards the growth of surviving microbes during storage (Siragusa, 1995). The treatment of pork carcasses by lactic acid reduced coliform counts and retarded (during five days' storage at 3°C) the onset of the logarithmic phase of their growth. Salmonellae were not detected on any samples (Pipek and Bacˇo, 1997). Decontamination of pork skin suspension with 1% lactic acid was effective for *Campylobacter jejuni* (Netten et al., 1994). Treatment with lactic acid eliminated *Salmonella typhimurium* from pork carcasses (Netten et al., 1995). Pathogens found in the environment of abattoirs (*Listeria monocytogenes* and *Y. enterocolitica*) may become adapted to lactic acid used to decontaminate meat. However they did not cause an increased health hazard, although the number of gram-negative spoilage organisms on pork skin was largely reduced by hot 2–5% lactic acid decontamination (Netten et al., 1997a). Lactic acid decontamination (1–5%

30–90 s) killed mainly gram-negative bacteria. During aerobic chilled storage after lactic acid decontamination the growth of gram-negative psychrotrophs was controlled only temporarily and these organisms became the dominant group of organisms (Netten et al., 1997b). Lactic acid decontamination of pork carcasses by dipping in 1–2% lactic-acid solutions brought a sharp decrease in the number of cfu of pathogens occurred on the skin of chilled pork belly cuts. Decontamination treatments applied during dressing of cattle carcasses were investigated for their effects on microbiological quality. Steam or hot pasteurization was shown to be consistently effective methods of reducing bacterial counts. Washing, followed by an effective pasteurization treatment, provided the maximal possible reduction in bacterial counts (Gill and Landers, 2003). James et al. (2000) compared potential methods for decontaminating lamb carcasses applied at 50 min postmortem for 8 s: steaming at 100°C, immersion in 90°C water and immersion in 90°C chlorinated water.

The steam system shows the best potential for industrial application due to its simplicity. The advantage of steam is explained by Kozempel, Goldberg, and Craig (2003). The surface will appear quite rough with many pores. It is difficult to kill bacteria that get into these pores with sanitizing solutions because surface tension prevents the liquid from entering the pores. Therefore, steam should be able to enter the pores and kill the bacteria. A very thin layer of air plus the entrapped moisture surrounds all solid food and steam cannot pass through these barriers to reach the bacteria. When vacuum is applied to the food to remove the air and moisture; and steam then rapidly applied to kill the bacteria in the pores, and then to expose the food to vacuum again to remove the condensate and evaporatively cool the surface. A process that exposes meat to vacuum, then steam, then vacuum again leads to the reduction of different pathogens by log 1.0– 2.0. For maximum effectiveness, the water temperature must be above 75°C (Siragusa, 1995).

The treatment of the pig carcass with water at 85°C for 20 s reduced the total numbers of bacteria by an order of 2 and these of *E. coli* by 2.5 as compared with untreated carcasses (Gill et al., 1995). The physical treatment by hot steam followed by spraying with lactic acid solution is another possibility for surface decontamination; see, for example, papers of Dorsa et al. (1996b) or Dorsa et al. (1996a). In this case, acid and heat inactivation of microorganisms follows release of microorganisms from the surface. The effect of combined treatment was recently proven by Kang et al. (2001). They observed that different combinations of hot water (82°C) and/or hot air (510°C) and lactic acid resulted in continuously decreasing microbial populations on the beef trim. Decontamination of swine carcasses by combination of rinsing with water and spraying with lactic-acid solution in commercial slaughterhouses was investigated. All treatment combinations effectively reduced microbial contamination (Sun-Jingxin et al., 2003). The treatment with lactic acid had only a negligible effect on the color (Pipek et al., 2004). In a study by Pipek et al. (2005), pig carcasses were decontaminated immediately after dressing at the end of the slaughter line, i.e., nearly 30 min postmortem. The decontamination treatment comprised hot steaming followed by spraying with the lactic-acid solution. Results from this study suggest that washing that includes lactic acid, followed by an effective pasteurization treatment (steam treatment) provides the maximal possible reduction in bacterial counts. This treatment reduced the microbial counts immediately after the treatment and retarded microbial growth during storage.

Time interval from sticking to chilling

The slaughtering and dressing process is performed at ambient temperature, while the carcass temperature is high. Thus, there is a great potential for an extensive growth of bacteria during the processing period. Most pathogenic bacteria of swine have a growth potential under these conditions, except for *Campylobacter* spp. which do not grow in an aerobic atmosphere. Provided that efficient cleaning and disinfection routines are used, the number of bacteria will drastically be reduced at the end of the production period. In environments not properly disinfected, additional growth will occur and a endemic flora may develop. Procedures for maintaining clean gloves, working clothes, tools and machines are especially important. The increase in numbers of bacteria on the carcass or in the environment may be predicted, using for example Food Micromodel (Food Micromodel Ltd. Leatherhead, Surrey, UK) taking into account environmental factors such as temperature and pH-value. The predicted lag period for S. *aureus* is 4 h at environmental conditions representing the carcass meat surface; for *Salmonella* spp. the corresponding lag period is 3 h (pH = 7.0; temperature = 30°C; NaCl on-water = 0.3%). Thus, the processing time in the slaughter hall and the time until proper chilling are crucial factors to be accounted for in HACCP/PHIS actions.

Chilling/Cold Storage

Normal chilling procedures are generally rapid chilling where the carcass surface temperature rapidly falls, followed by slower chilling. The chilling parameters vary from slaughterhouse to slaughterhouse. Maximal reduction in microbial growth occurs in slaughter houses that use blast chilling (−30°C to -10°C air, 1 - 1.5 h) followed by cold room storage (3-5X, overnight to 3 days). The effect of chilling on the potential growth of pathogenic bacteria may be predicted using models (Gill and Jones, 1992). During chilling, the number of *Campylobacter* spp. will be reduced due to a sensitivity to drying, freezing and aerobic atmospheres (Stern and Kazmi, 1989).

Bacterial growth will occur during storage of the pork. *A. hydrophila, L. monocytogenes* and Y. *enterocolitica* are reported to grow on meat stored at chill temperatures, but the growth rate is dependent on environmental factors such as temperature, pH-value and gaseous atmosphere (Palumbo, 1988; Luchansky and Doyle, 1991; Wallentin Lindberg and Borch, 1993). The growth may be limited by appropriate storage conditions such as storage temperature and type of packaging, and display conditions that do not permit growth of the identified bacterial hazards.

During the cooling of carcasses, the contaminating flora may proliferate, be contained or be reduced in numbers. If the chiller conditions allow carcass surfaces to remain moist and relatively warm for extended periods, then the psychrotrophic fraction of a flora will have the opportunity for substantial proliferation (Gill, 1982). Early cooling of carcass surfaces to low chiller temperatures will contain such growth, while surface drying associated with the cooling can result in decreasing numbers of the gram-negative fraction of the flora (Nottingham, 1982). In a study by Gill et al. (1992), those various microbiological results of carcass chilling were respectively observed at three different plants. Some growth was

apparent at plant A, where carcass surfaces at first cooled slowly. No growth was apparent at a second plant, where carcass surfaces were rapidly cooled at the beginning of the chilling operation by a blast of freezing air. At a third plant small batches of carcasses were loaded to a relatively large chiller that was also used for the storage of already chilled and packaged product. Those carcasses were thus well spaced in a chiller of refrigerative capacity well in excess of the heat load being imposed, in such circumstances, carcass surfaces readily dry (Gill, 1987). Surface drying would not be expected at larger plants, where management commonly seeks to limit the evaporative loss of carcass weight, and thus necessarily prevent extensive drying of the carcass surfaces.

One study noted a slight increase in the number of total viable counts during chilling, while other studies have noted decreases. Spoilage begins at a germ count of between 10^7 and $10^8/cm^2$ on the meat surface. Feldhusen et al. (1992) reported rapid chilling of carcasses at −5°C over a period of 40 minutes and then maintaining that temperature at +5°C was sufficient to reduce spoilage bacteria counts on pigskin at low atmospheric relative humidity of 80-90%, while after one week of cold storage at 100% relative humidity bacterial numbers increased to numbers required to begin spoilage. While it is recommended that carcasses be stored at the cold temperature (+5°C) with a low relative humidity (80-90%), it is known that low relative humidity leads to weight loss in carcasses (drying out of the meat), a compromise of 90% relative humidity is currently used (Tamm et al., 1976).

Packaging/Product Labeling

Permanent cooling of the air in the cutting rooms prevents *Salmonella* spp. colonizing certain ecological niches for longer periods (Berends et al., 1998). In the cutting room the area is wiped clean during breaks and at the end of the day. If cleaning is adequately done the area will be "*Salmonella* free." Throughout the day, once a contaminated carcass enters the processing line the number of contaminated carcasses will increase sharply to maximum levels (Berends, 1995). Berends et al. (1998) stated that based on data from a earlier study, during the first hour of production, the odds is a risk factor for cross contamination. The odds ratio of inadequate cleaning and disinfection of the line can be estimated at 12.8 at a 0.05% level of confidence, and the attributable risk at 0.67, meaning two thirds of cross contamination occurs during the first hour of production.

During the operations following dressing, i.e. chilling, cutting and deboning, a further spread of pathogenic bacteria will occur. The origin of the contamination may be either the carcasses or the environment. The contamination via carcasses should be limited if an effective HACCP/GMP-plan is in use. The contamination from the environment should also be limited by appropriate cleaning and disinfection routines.

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BEEF SLAUGHTER (03J)

The abattoir contains many environments that can lead to cross contamination with pathogens (USDA 1993, Belk 2001). Holding pens, slaughter and dressing processes, carcass skinning and evisceration have all been identified as points of entry for bacterial contamination (USDA 1993). Contamination is also possible from walls, floors, air, personnel, knives and protective garments (Fung et al.2001). Carcasses may even contaminate each other if they make direct contact (Elder et al.200). The extent to which carcasses are contaminated is directly influenced by plant design, the speed of slaughter and the overall skill of employees (Belk 2001). The season, type of animal being slaughtered and the specific site on the carcass can dramatically effect the level of contamination present on the carcass after slaughter (Sofos et al.1999).

Live Receiving/Pen Holding

Multiple strains of *E. coli* O157:h7 and *Salmonella* can colonize a single animal, or multiple animals from one farm, and these bacteria are shed in the feces (Faith et al., 1996, McEvoy et al., 2003). Animals are exposed to different strains during transport to feedlots or slaughter facilities. Observations from a survey of fecal shedding in cattle showed that calves tend to have a higher incidence of *E. coli* O157:H7 carriage than adults (Zhao et al., 1995), and shed greater numbers of bacteria for a longer period of time (Cray and Moon 1995). The prevalence of the bacterium in cattle is higher during the warmer months of the year, which correlates with the incidence of human disease (Hancock et al, 1994, Griffin 1995, Chapman et al., 1997). Ensuring that only clean, healthy animals are presented for slaughter and are processed correctly will reduce the incidence of contamination. In a study where contaminated hides were washed immediately prior to slaughter, contamination levels of carcasses contacted by a fecally soiled hide and those contacted directly by fresh feces were similar. This suggested that washing immediately before slaughter may not be the most effectual point in the process to address cleanliness of the animal (Bell 1997).

Stunning/Bleeding

The animal is directed out of the holding pen or taken off the truck via a chute to the "knock box" where it is stunned. Cross contamination of hides is possible as cattle fall to the floor or come into contact with sides of the chute after previously contaminated cattle have passed through. Additional contamination can occur if cattle emit feces or rumen contents at the knock box (Delazari et al 1998) or if dirty knives are used (Labadie et al. 1977).

Head Skinning and Removal

Cattle enter the main floor of the slaughter plant. Horns are removed using hydraulic cutters. The udder is removed, the and the head is skinned. The hide is cut down the midline, legs and front shanks.

Rodding the Esophagus/Hoof Removal

Proper tying of the esophagus to prevent the leakage of ingesta and to ensure that the gastrointestinal tract is removed without incident is essential to controlling contamination (Bell 1997).

Skinning and Related Operations

It is at this point that normally sterile muscle and fat tissues on the carcass surface are exposed to microbial contaminants. An individual carcass may be self- or cross-contaminated. If the carcass originates from an animal that is not infected, contamination may occur via aerosol diffusion or contact with contaminated equipment or a contaminated carcass. If the carcass originates from an infected animal, it may be self-contaminated via fecal or hide sources or cross-contaminated by the pathways described for non-infected animals. Meat becomes contaminated when feces or contaminated hides contact the carcass during slaughter (Gill et al.1995, Elder et al. 2000, Derfler 2004). The removal of the hide was identified as the chief source of contamination during slaughter and is a critical control point in beef slaughter HACCP plans. During processing, contamination spread to the carcass can range from 2 to 4 log cfu/cm^2 (Anderson et al.1980). *E. coli* O157:H7 was often present on the hide of animals following stunning, and cross contamination to the carcass was evident in that carcasses sampled immediately after dehiding were the most heavily contaminated (Elder et al.2000). The bulk of microbial contamination occurs during hide removal (Gill 1979, Bell 1997, Buchannan and Doyle 1997) from dust, dirt and fecal material that accumulate on the hide (Ayres 1955, Bell 1997). Cross contamination can occur via workers' gloves, knives, clothing, or during the changing of the hide-puller from one carcass to the next (Gill 1999).

Contamination at the hide puller can occur at several steps. For example, the tail can flip around and create aerosols (Getz 1999) or flip back on the carcass during hide removal. Aerosol can also occur when the hide separates from the carcass (Galland1997). Hide-removing machinery called up-pullers are possibly more like to cause aerosol contamination because the hide is being rolled up over the carcass rather than below it.

First decontamination

Following removal of the hide, one or more decontamination steps may be applied depending on the amount of visible foreign mater on the carcass. Knife trimming is used to remove visible spots of fecal decontamination greater than 1 inch in diameter. Spot vacuuming is used to remove visible spots of fecal contamination that are less than 1 inch in diameter. Increasingly, plants are rinsing carcasses with hot water and a variety of organic acids prior to evisceration. Any one of the three decontamination steps can reduce existing contamination on the carcass Bacon et al 1999, Galland 1997).

The effectiveness of knife trimming is highly variable (Prasai et al. 1995), and cross contamination through the knife cuts can occur if inadequate knife sterilization methods are used.

Sheridan et al. (1992) and Smeltzer et al. (1998) have identified equipment such as knives, gloves, and aprons as reservoirs of bacteria in the slaughterhouse.

Experimental studies have measured the reduction of *E. coli* on inoculated beef resulting from rinsing ingesta and manure from the carcass. Gill (1999) reported that carcass rinses reduced generic *E. coli* counts by 0.32 log CFU/cm^2. Dorsa et al. (1997) reported a 0.7 log CFU/ cm^2 reduction with a water rinse. Areas at risk of direct or indirect fecal contamination are the hock, inside leg, bung area and flank. While room temperature water washes are most effective at removing blood, hair, digesta and feces (Bell 1997), visual cleanliness of a carcass does not guarantee microbiological safety of the meat (Kriaa et al 1985). One study showed that rinsing carcasses with cold water could potentially redistribute microbial contamination over the carcass in a posterior to anterior direction (Bell 1997). This phenomenon has been previously demonstrated in other studies (Gill 1991, Hardin 1995).

Bunging

Bung tying is a possible source of contamination in the slaughter process, and great care must be taken to prevent bacterial transfer from the anus of the animal onto the edible adipose or muscle tissue (Gill et all.1995, McEvoy et al 2003b). The bung tying process involves cutting to loosen the anus, and then bagging the bung and securing with either a tie or a clip (FSIS 1994). The bung is then pushed through to the abdominal cavity, where it can be removed during evisceration (Romans et al.2001). Studies have shown that the bung tying operation reduces but does not eliminate the spread of pathogens to the carcass (Hudson et al. 1998). Tools or personnel that contact the bung may also contribute to cross contamination (McEvoy et al. 2003b). Cross contamination that is a direct result of manual bung tying may be eliminated by using an automated system. Such systems have reported lower total *E. coli* and coliform counts in the anal area than manual methods (Sheridan 1998).

Evisceration

During evisceration, the ventral midline of the carcass is split and the gastrointestinal tract is removed. The bung and esophagus must be tied off to prevent leakage and contamination, and the organs in the abdominal cavities must be removed. The gastrointestinal tracts of cattle can carry a multitude of enteric pathogens. The evisceration process carries the potential for ingesta contamination to the carcass, environment or equipment. To prevent contamination, great care must be taken to minimize the potential for evisceration defects, such as puncturing or rupturing the intestines (Hulebak and Schlosser 2001). Proper technique is critical to avoid contamination to the edible portion of the carcass (Aberle et al. 2001). If evisceration defects occur, corrective actions must be in place to remove any contamination from the carcass. Such measures include trimming of visible contamination, reducing the line speed so employees can exercise better caution and sanitizing tools (Hulebak and Schlosser 2001).

Carcass Splitting

At this step, the carcass is sawed in half, the tail is removed, and excess fat is trimmed away from each side. The carcass might become contaminated if a clean carcass comes into contact with contaminated machinery, hands, or other contaminated carcasses during splitting.

Second Decontamination

The second decontamination step occurs after carcass splitting. Different procedures for this decontamination step are used depending on the size of the plant.

Knife trimming of visibly contaminated meat occurs in both large and small plants after the carcass is split. Spot steam vacuuming may also be used in some plants.

Many plants have implemented at least two decontamination interventions, such as steam pasteurization and carcass rinses, that are effective in reducing pathogens on carcasses. The production of pathogen-free meat cannot be guaranteed (Dickson and Anderson 1992, Elder et al.2000) which is why the need for a decontamination step, in the form of washing and sanitizing, during slaughter is so important. Decontamination methods can improve the microbiological safety and increase shelf life and should be an integral part of the slaughter process (Dickson and Anderson 1992).

During the carcass rinse step, pathogens can be reduced or redistributed over the entire carcass (Bell 1997). The supplementation of hot water rinses with organic acids can increase effectiveness. Steam pasteurization of carcasses can significantly reduce contamination, if properly done (Gill 1998) Phebus et al. (1997) found a 3.53 log CFU/cm^2 reduction in *E. coli* 0157:H7 on inoculated carcasses. Gill (1998) reported up to a 2 log CFU/cm^2 reduction for generic *E. coli* from pasteurizing at 105.0C for 6.5 seconds. However, if the carcass was not clean and dry before steam pasteurization, there was little effect from the steam pasteurization. Kasner (1998) reported that steam pasteurization was effective in reducing *E. coli* O157:H7 only if the temperature was 93.3C (200degrees F) for 6 seconds or more. Phebus (FSIS Risk Assessment for *E .coli* O157:H7 in ground beef; personal communication) has suggested that the standard industry practice is to use 190 degrees F.

Chilling

Animals must be adequately spaced in the chiller to allow rapid cooling, but also to avoid carcass-to-carcass transfer of pathogens. Carcass sampling revealed that cross contamination does occur during chilling. Sampling of two carcasses before chilling resulting in one positive and one negative sample for *E. coli* O157:H7. However, after chilling, both carcasses tested positive for the organism. These carcasses were not together on the slaughter line, but were side by side during chill (McEvoy et al.2003b). Prompt chilling of carcasses after slaughter to below optimal bacterial growth temperatures is a critical measure, and chilling may affect the recovery of *E. coli* O157:H7 from carcasses

(Abdul-Raouf et al.1993: McEvoy et al.2003). It is recommended that carcasses are chilled 16-20 hours at -1 to -2 degrees C (Romans et al.2001). Chilling can reduce the prevalence of *E. coli* O157:H7 and will stress cells. The low temperature and water activity of the carcass may inhibit resuscitation (Stephens and Joynson 1998, Hara-Kudo et al.2000).

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POULTRY SLAUGHTER (O3J)

Live Receiving and Live Hanging

Live receiving is the initial step in the poultry slaughter process and begins when live poultry are received onto the official premise. Live hanging is the process of suspending live poultry in shackles after removing them from transport cages and begins when transport cages are off-loaded. With chemical immobilization, live poultry may be immobilized prior to hanging.

Potential risk factors

Biological potential risk factors exist during live receiving and live hanging and include pathogenic and non-pathogenic microorganisms on the feathers and skin, and in the crop, cecum, and colon contents of live poultry. *Salmonella* and *Campylobacter* are significant pathogens; psychrophilic microorganisms are significant spoilage organisms; and other microorganisms are indicators of sanitation process control.

Large numbers of microorganisms can be found on live poultry at live receiving. Kotula and Pandya (1995) found that 60.7% of feather samples and 41.8% of skin samples contained 6.7 $\log_{10}$ and 5.9 $\log_{10}$ *Salmonella*/gram respectively. Byrd et al. (1998) found *Campylobacter* spp. in 62% of crops and 4% of ceca. Wempe et al. (1983) recovered 3.8–4.8 $\log_{10}$ and 5.5 – 6.8 $\log_{10}$ *Campylobacter jejuni*/gm of feathers and cecal content respectively. Berrang et al. found more *Campylobacter* in feather (5.4 $\log_{10}$) than in skin (3.8 $\log_{10}$, $p \leq 0.05$) but other enterics did not differ at the two sites. Cloaca harbored more microbes (including *E. coli* and other coliforms) than any other site ($p \leq 0.05$). Kotula and Pandya (1995) found that 77.5% of feather samples and 57.5% of skin samples contained 7.4 $\log_{10}$ and 6.5 $\log_{10}$ *Campylobacter jejuni*/g. respectively. Geornaras et al. (1997) found 3.8 $\log_{10}$ *Pseudomonas*/gram of feathers. Mead et al. (1993) found 2-2.8 $\log_{10}$ *Pseudomonas*/gram neck skin . Kotula and Pandya (1995) reported that the feathers and. skin contained 7.9 $\log_{10}$ and 6.7 $\log_{10}$ *E. coli*/g. respectively.

Microorganisms present in/or live poultry at live receiving can cross-contaminate product. Bryan et al. (1968) demonstrated that *Salmonella* enters the establishment on incoming turkeys and contaminates equipment and subsequent poultry products. Clouser et al. (1995a) found that when *Salmonella* was present on the surface of turkeys prior to processing the incidence of *Salmonella* tended to increase throughout the slaughter process. Herman et al. (2003) concluded that establishments cannot avoid contamination when *Campylobacter jejuni*-positive poultry are delivered to live receiving. Furthermore, there is a statistically significant correlation ($p \leq 0.001$) between contamination of the carcass and presence of the microbe after processing. Berrang et al. (2003b) found that >50% of *Campylobacter*-negative broilers were *Campylobacter*-positive following exposure to feces in a commercial dump cage. Newel et al. (2001) demonstrated a link between *Campylobacter*-positive poultry at live receiving and *Campylobacter*-positive carcasses following immobilization, exsanguination, scalding, feather removal, evisceration, and

chilling. Fluckey et al. (2003) demonstrated a link between *Campylobacter*- and *Salmonella*-positive cecal content in live poultry and *Campylobacter*- and *Salmonella*-positive carcasses following evisceration and chilling. By using PFGE profiles, which allows identification of specific serotypes, whole carcasses were sampled at eight stages of turkey processing. Prevalence data showed that contamination rates varied along the line and were greatest after defeathering and after chilling. The same profiles were found to be present all along the processing line while on other occasions, additional serotypes were recovered that were not detected earlier on the line, suggesting that the birds harbored more than one serotype of Salmonella or there was cross-contamination occurring during processing (Nde et al., 2006). Chemical potential risk factors introduced at live receiving include violative chemical residues from a pharmaceutical, feed additive, pesticide, industrial compound, and/or environmental contaminate present within the edible tissue of live poultry. The USDA, FSIS monitors poultry products for the presence of chemical residues as part of its National Residue Program. Table 1 lists monitoring results from the 2003 National Residue Program monitoring results.

Table 1: National Residue Program Domestic Data (USDA, FSIS, OPHS, 2003)

	Sulfonamides			Arsenicals			Chlorinated Hydrocarbons			Avermectins & Milbemycins		
	N	P	V	N	P	V	N	P	V	N	P	V
Young Chicken	385			1087	579		476					
Mature Chicken	97	1		202	5		221	1				
Young Turkey	234			502	4		249	1				
Mature Turkey	234	2		97	1	1	214	5				
Ducks	95			336		1	248					
Geese	17			13			15					
Squab	20						22					
Ratite	5						10	5		7		
	N: number of analyses			P: number of non-violative positives			V: number of violations					

Controls

Biological and chemical potential risk factors present in or on live poultry received onto the official premise cannot be prevented, eliminated, or reduced to acceptable levels during live receiving or live hanging. However, they can be reduced through pre-harvest interventions. Berrang et al. demonstrated that when the level of microorganisms on live poultry at live receiving is high, the presence of microorganisms on raw product is high, and visa versa. Fluckey et al. (2003) found that the incidence of *Salmonella* and *Campylobacter* on the farm correlates with *Salmonella* and *Campylobacter* incidence during evisceration. Campbell et al. (1982) reported a 9% post-evisceration incidence of *Salmonella* from *Salmonella* free turkey flocks compared to 20% from non-*Salmonella* free

flocks. Producers can eliminate chemical potential risk factors through pre-harvest interventions that control pharmaceutical and chemical usage.

The National Chicken Council (NCC) (1992) and the National Turkey Federation (NTF) (2004) recommend that poultry producers implement pre-harvest sanitation and production practices shown to reduce hazards in edible poultry products. They recommend microbiological standards for feeds. Davies et al. (2001) and Corry et al. (2002) traced *Salmonella* serotypes recovered from the farm and during transportation back to the feed mills.

The NCC and NTF also recommend bio-security, maintenance, and sanitation programs for facilities and equipment to reduce pathogenic and non-pathogenic microorganisms in/on live poultry prior to live receiving. Davies and Wray (1996) identified rodents and faulty application of disinfectants as causes for the persistence of *Salmonella* in growing houses. Herman et al. (2003) identified employee clothing as the source of *Campylobacter*-positive flocks. Evans and Sayers (2000) identified important factors for preventing *Campylobacter* infection in a flock including: buildings in good repair, boot dips, high standards of cleaning, and disinfecting drinking water. Higgens et al. (1981) demonstrated that failure to clean and disinfect air inlets and fans contributed to recontamination of facilities with *Salmonella*. The microbial composition of the air in a high-throughput chicken-slaughtering facility was examined by sampling various areas. It was found that the highest counts of microorganisms were recorded in the initial stages of processing, comprising the receiving-killing and defeathering areas, whereas counts decreased toward the evisceration, air-chilling, packaging, and dispatch areas (Lues et al., 2007). Rose et al. (2000) identified the lack of cleaning and disinfection between flocks as a significant risk factor for the persistence of *Salmonella*. Corry et al. (2002) and Slader et al. (2002) linked failure to clean and sanitize transport crates with *Campylobacter*- and *Salmonella*-positive poultry being received onto the official premise during live receiving.

The NCC and NTF further suggest proper feed and water withdrawal to minimize fecal and ingesta contamination during processing. Wabeck (1972) recommended taking broilers off feed and water eight to ten hours prior to slaughter. Bilgili (1988) found that decreasing feed withdrawal times increased the likelihood of gastrointestinal breakage during processing. Northcutt et al. (2003) determined that increasing feed withdrawal to 12 hours increased *Campylobacter* and *Salmonella* levels in post carcass rinses 0.4 $\log_{10}$ CFU/ml and 0.2 $\log_{10}$ CFU/ml respectively. Bilgili and Hess (1997) found that feed withdrawal periods $\geq$14 hours increased intestine and gallbladder fragility, which increased fecal and bile contamination during evisceration. Hinton et al. (2000, 2002) found that providing broilers with a 7.5% glucose solution or a sucrose solution during feed withdrawal decreased the crop pH, increased the level of lactobacillus, and decreased the incidence of *Salmonella typhimurium* in the crop during feed withdrawal ($p \leq 0.05$). Line et al. (1997) found that feeding *Saccharomyces boulardii*, a non-pathogenic yeast, to broilers during feed withdrawal reduced the incidence of *Salmonella* in the cecum during to crating and transport. Acidifying the drinking water at the time of feed withdrawal may help also to reduce levels of *Salmonella* in incoming birds. Byrd et al. (2001) found that administering organic acids at the time of feed withdrawal maintained a more acidic pH in the crop and

provided birds with an alternative to consuming potentially contaminated litter. Offering birds an organic acid in the water significantly lowered post-harvest crop contamination with *Salmonella* ($p \leq 0.001$) and *Campylobacter* ($p \leq 0.001$). This type of treatment could be a cost-effective approach that does not require radical changes in current management practices. Byrd et al. (2003) suggested that sodium chlorate added to the water at the time of feed withdrawal could significantly reduce levels of *Salmonella* in the crop and ceca.

Feed withdrawal may, however, affect the intestinal integrity due to depletion of intestinal mucus (Thompson and Applegate, 2006) as well as reduction of digestive tract mass (Nijdam et al., 2006), which can increase susceptibility to infection. Recent studies suggested that special diets could be a good substitute for the feed withdrawal period held before transportation to the processing plant. Special diets that show favorable results include semi-synthetic feed with high carbohydrate concentration (Delezie et al., 2006) or a commercial whole wheat diet (Rathgeber et al., 2007). Alternatively, a commercial whole wheat diet fed prior to feed withdrawal eliminated the deleterious effects on gut weight and content (Delezie et al., 2006).

In addition to biosecurity measures, producers have other means of reducing *Salmonella* in poultry flocks. Vaccinations, especially those against *Salmonella enteritidis*, reduce shedding of the organism in the intestine as well as in organs including the ovaries, theoretically decreasing the contamination of subsequently laid eggs (Davison et al., 1999). Reducing intestinal colonization and consequently fecal shedding of *Salmonella enteritidis* could provide two-fold protection by reducing both vertical as well as horizontal transmission (Gast et al., 1993). After infection with S. enterica serovars *typhimurium* or *enteritidis,* the high titers of Salmonella-specific antibodies achieved has been shown to demonstrate a high degree of cross-reactivity against other serovars (Beal and Smith, 2007). Furthermore, live attenuated vaccines given to very young chicks have been shown to provide protection through the "colonization-inhibition effect". Because a chick's gut is devoid of microbial flora, there is extensive multiplication by the vaccine, making it difficult for pathogenic organizations to become established (Barber et al., 1999). Autogenous bacterins are important interventions and the poultry industry has petitioned the Animal Plant Inspection Service (APHIS) to rewrite the regulations to allow autogenous vaccines use.

Prebiotics and probiotics are established treatment alternatives for reducing *Salmonella* in poultry. Gibson and Roberfroid (1995) define prebiotics as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one of a limited number of bacteria in the colon" Fuller (1989) defines probiotics as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal balance". It is believed that prebiotics and probiotics act as dietary resources that might be instrumental in stabilizing gut flora as well as helping to prevent pathogenic organisms from colonizing the gut and causing disease (Holzapfel et al., 1998). Tellez et al. (2001) found that significantly less *Salmonella enteritidis* was isolated from the cecum as well as from tissue organs in birds treated with a Avian Pac Plus® that contained probiotics as well as egg-source antibodies for *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella heidleberg* as compared to untreated controls. Netherwood et

al. (1999) found that once probiotics were discontinued, the microflora returned to levels found in untreated controls, suggesting that probiotics do not become established in the gut and continued use is required.

Other interventions that show promise are yet to be implemented. As the potential risk factor over antibiotic resistance increases, there has been renewed interest in exploiting the antibacterial properties of bacteriophages and bacteriocins. More effective vaccines may eventually come marketed within bacterial ghosts. Richardson et al. (2003) experimented with electric space charges as a means of reducing airborne transmission of bacterial pathogens. The poultry industry has continued interest in using undefined competitive exclusion (CE) products. Because undefined CE products make therapeutic claims, the Food Drug and Administration (FDA) classifies them as drugs. Since the FDA does not recognize these products as either safe or effective, it has labeled them as unapproved new drugs. The FDA did approve a defined CE product, PREMPT®, which has since been removed from the market. A recent study which included 118 commercial turkey hen lots, ranging from 1,542 to 30,390 hens per lot, of either Nicholas or Hybrid genetic lines was conducted to look at the effect of a selected commercial Lactobacillus-based probiotic (FM-B11) on turkey body weight, performance, and health. When each premise was compared by level of performance as good, fair, or poor (grouping based on historical analysis of 5 previous flocks), the probiotic appeared to increase the performance of the poor and fair farms ($p \leq 0.05$) (Torres-Rodriguez et al., 2007).

Of the interventions discussed, not one alone is capable of eliminating pathogens. Interventions vary in their effectiveness for both researchers as well as producers. Some appear to have synergistic effects when used in combination. More research as well as application is needed to resolve these issues.

Immobilization and Exsanguination (Bleeding)

Immobilization renders live poultry unconscious in preparation for exsanguination (bleeding); however, death by slaughter can occur unintentionally or by design. Immobilization begins when the immobilizing agent is applied and ends when the cervical vessels are severed. Immobilization methods are classified as mechanical, chemical, and electrical and should be implemented in accordance with good commercial practices in a manner that will result in thorough bleeding of the carcasses.

Mechanical immobilization is impractical in large poultry establishments. However, it is useful in emergencies or to immobilize small numbers of live poultry, which makes it a practical method in small and very small establishments. Decapitation, cervical dislocation, and blunt trauma to the head are the most common forms of mechanical immobilization.

Chemical immobilization exposes live poultry to a gas individually in boxes or tunnels, or in batches. The most common gases are CO_2 (Drewniak et al., 1955; Kotula et al., 1961) and argon (Raj and Gregory, 1990, 1994). When chemical methods are used, live poultry may be immobilized prior to live hanging.

Electrical immobilization is the most common method in use worldwide. It is the best method of achieving rapid brain failure and the cheapest and most effective method of poultry slaughter. The EEC recommends electrical immobilization with a minimum of 120 mA to instantaneous render poultry unconscious, effect ventricular fibrillation, and produce death by slaughter (Fletcher, 1999). A majority of U.S. poultry processors utilize low voltage, high frequency methods (Fletcher, 1999; Heath et al., 1994). The remaining U.S. processors utilize high voltage with no specified waveform. Gregory and Wooton (1986) determined that low voltage immobilization with 30-60 V, 20-45 mA does not result in death by slaughter, while high voltage stunning with 150V, 100 mA induces ventricular fibrillation and death by slaughter. Both systems accomplish the desired end result. Kuenzel et al. (1978) determined that 50V/60Hz circuits are 35% more cost effective than 100V variable frequency circuits and 225% more cost effective than DC circuits. However, Kuenzel and Walther (1978) concluded that DC currents are safer and improve exsanguination time compared to AC circuits because blood is not shunted from peripheral to central blood vessels. A recent study examined different slaughter techniques to determine their effects on pH (24 h), color (24 h), lipid oxidation, residual hemoglobin concentration (24 h), and sensory evaluation (d 1 and 4 postmortem) in broiler breast fillets and concluded that the electrical stunning and decapitation method had the most favorable results for sensory quality regardless of whether the chickens were pre-bled (Alvarado et al., 2007).

Exsanguination guarantees death by slaughter and ensures that poultry have stopped breathing prior to scalding. Exsanguination begins when the cervical vessels are severed and ends when the carcass enters the scald process. For exsanguination to cause death by slaughter, it is important that the cervical vessels be cut promptly and efficiently so that poultry do not regain consciousness and/or enter the scald tank before they have stopped breathing.

Potential risk factors

Biological potential risk factors include cross-contamination with pathogenic and nonpathogenic microorganisms. Immobilization (Mead et al., 1994) can void feces and further contaminate the carcass exterior, scald tank water, and feather removal equipment. Papa and Dickens (1989) found that 53% of broilers produced an average excretion of 1.5 g during electrical immobilization and that the volume of the excretion increased as feed withdrawal time increased. Musgrove et al. (1997) found that *Campylobacter* in whole carcasses rinses increased 0.5 $\log_{10}$ CFU/ml following electrical immobilization. Mead et al. (1994) found that the physical pressure of the killing knife against the carcass can void crop content with similar affect.

Trim nonconformance is an undesirable side effect of immobilization. Raj (1994) and Raj et al. (1990) identified a link between electrical and chemical immobilization and hemorrhage and broken bones in turkeys and broilers. Chemical immobilization results in a lower incidence of trim nonconformance compared to electrical immobilization (Raj and Nute, 1995; Raj et al., 1997, 1998). Grossly significant hemorrhages can interfere with accurate post mortem disposition.

Failure to properly exsanguinate can result in poultry entering the scald tank before breathing has stopped. Heath et al. (1981) speculated that red discoloration of the skin results when live poultry enter the scald tank. Heath et al. (1983) later concluded that poultry entering the scald tank alive develop red discoloration of the skin, that the discoloration is confined to the pterylae, and that the apteria is never discolored. Griffiths (1985) demonstrated that only poultry entering the scald tank alive result in red discoloration of the skin. Poultry that are dead, either by slaughter or by other causes, when they enter the scald tank, do not result in red discoloration of the skin. Griffiths further demonstrated that the red discoloration is due to marked peripheral vascular dilation of blood vessels in the skin and subcutis.

Controls

Biological and chemical potential risk factors present during immobilization and exsanguination cannot be prevented, eliminated, or reduced to acceptable levels during these process steps. However, they can be influenced through pre-harvest interventions and choice of processing method.

Feed withdrawal time influences the incidence of feces voided during immobilization. Papa and Dickens (1989) found that only 8%, 42%, 50%, and 58% of broilers produced an excretion when the feed withdrawal time was 4, 8, 12, and 16 hours respectively. McNeal et al. (2003) found that exsanguination by decapitation following electrical immobilization produced less wing flapping, body motion, and quivering because decapitation kills poultry quicker than severance of the cervical vessels.

Scalding

Scalding begins when the poultry carcass enters the scald system and ends when feather removal commences. Scalding prepares the carcass for feather removal by breaking down the proteins holding feathers in place and opening up feather follicles.

Variables requiring consideration during the scald process step are mechanical, physical, and chemical. Mechanical variables include counter-current flows and agitation to produce a washing effect. Counter current systems move water counter to the direction of poultry carcasses at all points. Water enters the system at the point where poultry carcasses exit and the water exits at the point where poultry carcasses enter, producing a dirty-to-clean gradient that continually moves poultry carcasses into cleaner water. Cleaner water is a relative condition as the amount of dry matter and microorganisms in the scald water increase over time. Physical variables are time and temperature, which influence washing and antimicrobial effects. The chemical variable is pH, which also influences the antimicrobial effect.

Immersion scalding is the most common scald technology in use and is best described as dragging carcasses through a tank of hot water. Immersion systems come in single- and

multi-stage configurations, incorporating mechanical and physical variables. Single-stage systems provide less washing effect than multi-stage systems.

U.S. poultry processors prefer a "hard scald" combining shorter scald times and higher scald temperatures. A "hard scald" facilitates removal of the epidermis, which enhances the adhesion of coatings commonly used with fried foods. European poultry processors prefer a "soft scald" combining longer scald times and lower scald temperatures. A "soft scald" retains much of the epidermis and natural skin color.

Common Scalding Times and Temperature for Various Classes of Poultry		
Broilers (hard scald)	30-75 seconds	59-64° C
Broilers (soft scald)	90-120 seconds	51-54° C
Turkeys	50-125 seconds	59-63° C
Quail	30 seconds	53° C
Waterfowl	30-60 seconds	68-82° C

Steam-spray scalding is a less popular alternative. Klose et al. (1971), Kaufman et al. (1972) and Dickens (1989) found that a mixture of steam and air at 50-60° C and 137.9 kPa pressure applied for approximately two minutes provided a uniform scald of either dry or damp broilers, facilitated feather removal, and yielded carcasses microbiologically equivalent to immersion systems. Some religious dietary laws prohibit scalding and soak poultry carcasses in cold water.

Potential risk factors

Biological potential risk factors include pathogenic and non-pathogenic microorganisms introduced during the scald process. These microorganisms are present on the internal and external surfaces of the carcass as well as in the scald water.

Salmonella and *Campylobacter* are the most common pathogenic microorganisms identified with the scalding process step. Berrang et al. (2000a) recovered 5.4 $\log_{10}$, 3.8 $\log_{10}$, 4.7 $\log_{10}$, 7.3 $\log_{10}$, and 7.2 $\log_{10}$ *Campylobacter*/gm from feathers, skin, crop content, cecal content, and colon content prior to scalding. Georgnaras et al. (1997) isolated *Salmonella* from 100%, *Listeria* spp. from 33%, and *Staphylococcus aureus* from 20% of skin and feather samples collected prior to scalding. Cason et al. (2000) found that 75% of scald tank water samples were *Salmonella*-positive and recovered an average of 10.9 MPN *Salmonella*/100 ml, or about 1 *Salmonella* bacteria/9 ml. They found significantly lower prevalence of microorganisms with increasing passes between tanks but removal of coliforms and *E. coli* is more effective ($p \leq 0.02$) than *Salmonella*. Wempe et al. (1983) recovered an average of 1.6 $\log_{10}$ *Campylobacter jejuni* CFU/ml from scald tank water.

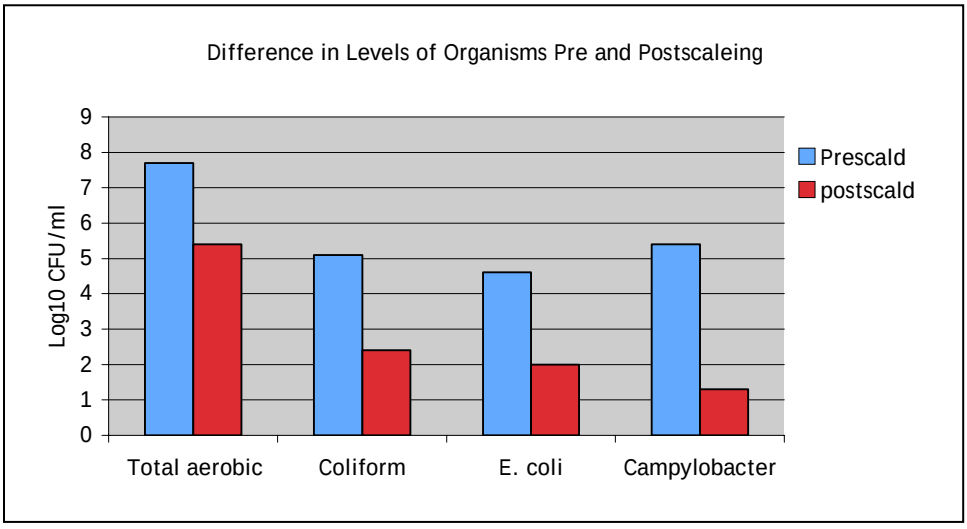
Because scalding washes much of the dirt and feces off of the carcass exterior, more microorganisms can be removed during scalding than during any other process step. Georgnaras et al. (1997) found a 38% decrease in *Salmonella*-positive carcasses. Acuff et al. (1986) reported a 312 MPN/100 cm^3 decrease in *Campylobacter jejuni* on turkey skin. Berrang and Dickens (2000) reported a 2.9-4.1 $\log_{10}$ reduction in *Campylobacter*/ml in

carcass rinses. Lillard (1990) found a 1.1 $\log_{10}$ and 1.5 $\log_{10}$ CFU/ml decrease in aerobic bacteria and Enterobacteriaceae respectively in carcass rinses. Geornaras et al. (1997) found a 1.0 $\log_{10}$ CFU/g decrease in *Pseudomonas* spp. in skin samples. Berrang and Dickens (2000) reported 2.1 $\log_{10}$ and 2.2 $\log_{10}$ CFU/ml reductions in coliforms and *E. coli* respectively in carcass rinses.

However, Berrang et al. (2003a) found that immersion scalding increased aerobic bacteria 0.9 $\log_{10}$ CFU/ml, coliforms 0.8 $\log_{10}$ CFU/ml, *E. coli* 1.5 $\log_{10}$ CFU/ml, and *Campylobacter* spp. 0.8 $\log_{10}$ CFU/ml in lungs rinses taken from broilers indicating that microorganisms were added to the respiratory tract during immersion scalding. These microorganisms carry forward into subsequent processing steps. In contrast, Kaufman et al. (1972) found that the air sacs of steam-scalded broilers contain 3 $\log_{10}$ fewer microorganisms than the air sacs of immersion-scalded broilers. The number of microorganisms on poultry carcasses exiting the scald tank is relative to the number of microorganisms in or on the poultry carcass entering the scald tank. The scald process cannot eliminate excessively high numbers of microorganisms entering the process.

A disadvantage of washing dirt and feces off of the exterior carcass surface is the accumulation of microorganisms in the scald water making the scald tank a source of cross-contamination for subsequent carcasses. Mulder et al. (1978) recovered a marker organism introduced prior to scalding from the 230th carcass exiting the scald. Cason et al. (1999) determined that the 4.2 $\log_{10}$ aerobic bacteria/ml, 2.7 $\log_{10}$ *E. coli*/ml, and 2.9 $\log_{10}$ *Campylobacter*/ml of carcass rinse present on carcasses post-feather removal originated from the scald process.

The following chart illustrates the reduction in microorganisms that occurs during the immersion scalding process step. For each microorganism considered, Berrang and Dickens (2000) and Berrang et al. (2003a) measured a reduction in the mean $\log_{10}$ CFU/ml of whole carcass rinse taken from broiler carcasses pre- and post-immersion scalding *($p \leq 0.05$ for all of the organisms tested).*



Chemical potential risk factors include residues introduced during the scald process through the excessive application of technical processing aids and/or antimicrobial agents. Technical processing aids enhance the scalding process and include surfactants, denuding agents and emollients. Surfactants reduce surface tension, improve wetting agent function, and inhibit foam. Alkaline denuding agents loosen the keratinized outer layer of the epidermis. Emollients retain moisture and prevent excessive drying of the denuded dermis. Many of these chemicals are generally regarded as safe (GRAS) by the FDA. Others are listed with restriction in the Code of Federal Regulations; 9 CFR 424.21, "Use of food ingredients and sources of radiation". When a processing aid produces the same technical effect at lower scald water temperatures, a greater number of microorganisms can survive the scald process.

Controls

Biological and chemical potential risk factors cannot be prevented or eliminated during the scald process step; however, they can be reduced.

The National Chicken Council (NCC) (1992) and Waldroup et al. (1992) identified counter current systems, sufficient water replacement with, and a post-scald carcass rinse as good manufacturing practices for efficient immersion scalding. Waldroup et al. (1993) found that counter current scalding reduced aerobic bacteria, coliform, and *E. coli* 0.64 $\log_{10}$, 0.76 $\log_{10}$, and 0.72 $\log_{10}$ CFU/ml respectively, and *Salmonella* prevalence by 10% in scald water. James et al. (1993) found that counter- current scalding combined with a carcass rinse reduced aerobic bacteria, Enterobacteriaceae, and *E. coli* 0.68 $\log_{10}$, 0.37 $\log_{10}$, and 0.08 $\log_{10}$ CFU/carcass respectively, and the incidence of *Salmonella*-positive carcasses by 3%. Multi-tank immersion systems further improve the microbiological quality of the scald water. In a three stage counter current system, Cason et al. (2000) reported a reduction in coliforms from 3.4 $\log_{10}$ to 2.0 $\log_{10}$ to 1.2 $\log_{10}$ CFU/ml and in *E. coli* from 3.2 $\log_{10}$ to 1.5 $\log_{10}$ to 0.8 $\log_{10}$ CFU/ml in tanks 1, 2, and 3 respectively ($p \leq 0.05$). Cox et al. (1974) determined that one minute of agitation reduced aerobic bacteria on broiler skin by 0.42 $\log_{10}$ CFU/cm^2 after one minute.

Failure to maintain a proper time/temperature combination diminishes the desired technical effect of preparing feathers for removal and detracts from sanitary dressing. High scald temperature can cause the carcass to become oily, which favors the retention of microorganisms on the carcass surface. Cox et al. (1974) determined that immersion in hot water for one minute reduced aerobic bacteria 0.91 $\log_{10}$ CFU/cm^2. Yang et al. (2001) found that a 5-minute exposure at 50-60°C produced reductions of 3.8 $\log_{10}$ *Campylobacter jejuni*/ml and 3.0 $\log_{10}$ *Salmonella typhimurium*/ml in the scald tank water; and 1.5 $\log_{10}$ *Campylobacter jejuni*/ml and 1.3 $\log_{10}$ *Salmonella typhimurium*/ml on chicken skins.

Immersion scalding produces a relatively smooth, microbiologically superior skin surface compared to steam-spray and kosher methods that result a highly wrinkled micro-topography that facilitates attachment of microorganisms. Kim and Doores (1993) concluded that the incidence of *Salmonella*-positive turkey carcasses is higher with kosher

processing due to trapping of *Salmonella* in the keratinized epithelium. Lillard (1989) concluded that microorganisms become entrapped in ridges and crevices that become more pronounced in skin following immersion in water and are less accessible to antimicrobial treatments. Clouser et al. (1995b) recovered *Salmonella* from 57% of steam-spray and 37% of kosher skin samples compared to 23% with conventional methods.

Within 120 minutes of the start of operations, the dissociation of ammonium urate from poultry feces to uric acid and ammonium hydroxide can reduce scald water pH from 8.4 to 6.0 (Humphrey, 1981). The protein and minerals in the scald tank water then act as a buffer to maintain this pH for the rest of the working day. *Salmonella typhimurium* and *Salmonella newport* are most heat resistant at pH 6.1 (Okrend et al., 1986), *Campylobacter jejuni* at 7.0 (Humphrey and Lanning, 1987), *Aerobacter aerogenes* at pH 6.6 (Strange and Shon, 1964) and *Streptococcus feacalis* at pH 6.6 (White, 1963). Hydrogen ion concentration influences the rate of endogenous RNA degradation and a shift in pH away from optimal, while probably not the primary cause of microbial death in scald water, increases RNA degradation, hinders microbial metabolism, and contributes to microbial death.

Increasing scald water pH reduces microbial levels in the water. When scald water pH was increased from 7 to 9, Humphrey and Lanning (1987) determined that the time needed to achieve a 1-$\log_{10}$ reduction in *Campylobacter jejuni* was reduced from 11½ to 2 minutes; *Salmonella* levels were reduced from 13.9 MPN/100 ml to 3 MPN/100 ml; and the incidence of *Salmonella*- and *Campylobacter*-positive water samples from 100% to 26%. When scald water pH was adjusted to 9 after four hours of production and maintained for the remainder of the day, Humphrey et al. (1984) determined that aerobic bacteria and Enterobacteriaceae levels decreased by 0.4 $\log_{10}$ CFU/ml and 0.5 $\log_{10}$ CFU/ml respectively; and the death rate of *Salmonella typhimurium* attached to the skin increased 57%. Lillard et al. (1987) reported that reducing scald water pH to 3.6 by the addition of 0.5% acetic acid decreased aerobic bacteria 2.2 $\log_{10}$ CFU/ml in scald water.

The same can be said for decreasing scald water pH. Okrend et al. (1986) determined that reducing scald tank water pH to 4.3 by the addition of 0.1% acetic acid increased the death rate of *Salmonella newport* and *Salmonella typhimurium* 91%. However, the same is not true for microorganisms on the surface of poultry carcasses. Humphrey and Lanning (1987) reported that scalding at pH 9.0 had no affect on the incidence of *Salmonella* and *Campylobacter* on broiler carcasses. Lillard et al. (1987) found that reducing scald water pH to 3.6 did not reduce aerobic bacteria or Enterobacteriaceae on carcass surfaces. It is important to understand that these reductions take place in the scald tank water and not on the carcass surface.

Feather Removal

Feather removal eliminates the feathers and stratum corneum in preparation for evisceration. Feather removal begins when carcasses enter the feather removal equipment and continues until the exterior surface of the poultry carcass is free of feathers and cuticle. Feather removal technology is fairly uniform across the poultry industry. Carcasses pass

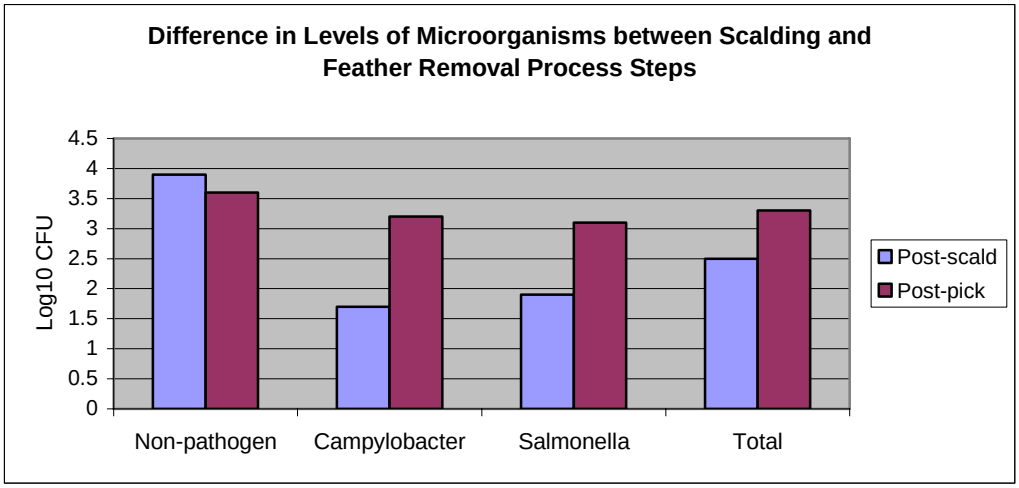
through one or more pieces of equipment that remove feathers by the mechanical action of rubber picking fingers beating against the carcass. Most establishments utilize a continuous process; however, batch processes are common in small, low volume establishments. Some very small establishments rely on manual methods to remove feathers. Following mechanical feather removal, goose carcasses are immersed in molten wax and dipped in ice water to facilitate removal of the down feathers. The hardened wax is manually removed, taking the down feathers with it.

Potential risk factors

Biological potential risk factors include pathogenic and non-pathogenic microorganisms introduced during the feather removal process. These microorganisms are present on the internal and external surfaces of the carcass as well as on the feather removal equipment and increase as an unavoidable consequence of the process. *Salmonella* and *Campylobacter* are the most common pathological microorganisms identified with the feather removal process. Acuff et al. (1986) determined that regardless of the number of *Campylobacter jejuni* present on turkey carcasses entering the establishment, on average, *Campylobacter jejuni* increased 150 MPN/100 cm^3 during feather removal. Izat et al (1988) found that feather removal increased *Campylobacter jejuni* on broiler carcasses 1.7 $\log_{10}$ CFU/1000 cm^3. Abu-Ruwaida et al. (1994) reported that *Campylobacter* and *Staphylococcus aureus* levels rose 1.6 $\log_{10}$ CFU/gm and 0.30 $\log_{10}$ CFU/gm, respectively, and the incidence of *Salmonella* was 100% post-feather removal. Berrang and Dickens (2000) found that *Campylobacter* in whole carcass rinses increased 1.9-2.9 $\log_{10}$ CFU/ml and that *Salmonella* (Berrang et al., 2001) on breast swabs increased 1.2 $\log_{10}$ CFU/cm^3.

Clouser et al. (1995a) found a >200% increase in *Salmonella*-positive of turkey carcasses after feather removal and concluded that when *Salmonella* is present prior to feather removal the incidence of *Salmonella* tends to increase throughout evisceration and chilling. Georgnaras et al. (1997) isolated *Salmonella* from 100% of carcasses following feather removal. The feather follicle has been implicated as a harborage for microorganisms. However, Cason et al. (2004) found no statistically significant difference ($p>0.05$) in aerobic bacteria, *E. coli*, and *Campylobacter* levels between feathered and featherless birds and concluded that microbial adhesion, not harborage in follicles, is the mechanism behind microorganisms present on poultry skin.

The following table summarizes data compiled from various authors cited in this document and illustrates the increase in biological potential risk factors during feather removal.



Within the feather removal equipment, the rubber picking fingers and recycled water are sources of cross-contamination. Georgnaras et al. (1997) isolated *Salmonella* from 33% the picking fingers. Wempe et al. (1983) recovered an average of 3.88 $\log_{10}$ *Campylobacter jejuni*/ml from 94% of feather removal water samples. Whittemore and Lyon (1994) recovered 5.46-5.73 $\log_{10}$ *Staphylococcus* spp., 5.83-6.04 $\log_{10}$ aerobic bacteria, and 5.05-5.44 $\log_{10}$ Enterobacteriaceae from the rubber picking fingers. Mead et al. (1975) and Allen et al. (2003b) found that a marker organism inoculated onto post-scalding carcasses dispersed for ≤200 carcasses via the feather removal. Mulder et al. (1978) found that a marker organism introduced prior to feather removal could be recovered from the 580th carcass exiting the feather removal equipment. Georgnaras et al. (1997) attributed increases of 1.1 $\log_{10}$ aerobic bacteria/g, 0.9 $\log_{10}$ Enterobacteriaceae/g, and 3.1 $\log_{10}$ *Pseudomonas* spp./g in neck skin samples following feather removal to the action of the rubber picking fingers.
Allen et al. (2003a) concluded that feces forced out of the cloaca by the action of picking fingers against the carcass cross-contaminated adjacent carcasses. Berrang et al. (2001) found that the incidence of *Campylobacter*-positive carcass rinses decreased 89% and *Campylobacter* levels decreased 2.5 $\log_{10}$ CFU/ml when the escape of feces from the cloaca was prevented. Buhr et al. (2003) confirmed the result, finding that plugging the cloaca decreased *Campylobacter*, coliforms, *E. coli*, and aerobic bacteria 0.7 $\log_{10}$, 1.8 $\log_{10}$, 1.7 $\log_{10}$, and 0.5 $\log_{10}$ CFU/ml respectively in rinse samples.

A clear demonstration for the role of fingers in cross contamination was shown by means of molecular characterization. Salmonella subtypes found on the fingers of the picker machines were similar to subtypes isolated before and after defeathering, indicating that the fingers facilitate carcass cross contamination during defeathering (Nde et al., 2007). Similar conclusions were made for cross contamination of *Campylobacter spp*. Using molecular profiling (Takahashi et al., 2006) in a poultry plant in Japan.

Airborne microorganisms have been implicated as a source of cross-contamination during feather removal. Whyte et al. (2001a) recovered 12.7 $\log_{10}$ *Campylobacter* per 15 ft^3 of air in broiler and hen establishments. Northcutt et al. (2004) recovered 1.5 $\log_{10}$ Enterobacteriaceae/ml of air during commercial processing of Japanese quail. Lutgring et

al. (1997) recovered 2.5-6 $\log_{10}$ psychrophilic bacteria/m^3 in turkey and duck processing establishments. However, Berrang et al. (2004) found that exposing *Campylobacter*-negative broiler carcasses to air near feather removal equipment for 60 seconds only increased *Campylobacter* 0.20 $\log_{10}$ CFU/ml in carcass rinses and concluded that airborne contamination does not contribute to high levels of *Campylobacter* routinely found on broiler carcasses after feather removal (95%CI).

Controls

Biological hazards and potential risk factors cannot be prevented, eliminated, or reduced to acceptable levels during feather removal.

The National Chicken Council (1992) and Waldroup et al. (1992) recommend preventing feather buildup, continuous rinses for equipment and carcasses, and regular equipment adjustment to minimize cross-contamination.

Changes in technique and/or equipment can affect microbial numbers on equipment and product. After increasing the number of rubber feather removal fingers, decreasing chlorine levels, and increasing cabinet temperature, Purdy et al. (1988) found that *Staphylococcus aureus*, coliforms, and Enterobacteriaceae on the feather removal fingers increased by 3.2 $\log_{10}$.CFU, 2.0 $\log_{10}$.CFU, and 4.6 $\log_{10}$.CFU respectively and *Staphylococcus aureus*, coliforms, and Enterobacteriaceae on the poultry skin samples increased by 2.8 $\log_{10}$.CFU, 5.0 $\log_{10}$.CFU, and 5.6 $\log_{10}$.CFU respectively. Allen et al. (2003a) determined that increasing the distance between carcasses and water curtains at the entrance and/or exit of the feather removal cabinet had no affect on cross-contamination. Clouser et al. (1995a) concluded that when aerobic plate counts are high at the start of feather removal, they remain proportionately high throughout processing.

Interventions applied during feather removal have yielded mixed results. Berrang et al. (2000b) concluded that rinsing carcasses with 71°C water for 20 seconds post-feather removal spraying had no significant effect on microbial contamination. Mead et al. (1975) found that a 10-20 ppm available chlorine carcass rinse did not reduce carriage of a marker organism on turkey carcasses passing through the feather removal equipment and contributed the result to inadequate contact time. Later, Mead et al. (1994) found that an 18-30 ppm available chlorine rinse reduced carriage of a marker organism on hen carcass passing through the feather removal equipment. Dickens and Whittemore (1997) found that a 1% acetic acid rinse post-feather removal reduced aerobic bacteria 0.6 $\log_{10}$ CFU/ml in whole carcass rinse without altering carcass appearance; but a similar application of 0.5%-1.5% hydrogen peroxide caused bleaching and bloating of carcasses.

Evisceration

Evisceration removes the internal organs and any trim/processing defects from the carcass in preparation for chilling. The technology varies widely across the poultry industry but always includes the following basic process steps.

- Remove the crus

- Remove the oil gland
- Sever the attachments to the vent
- Open the body cavity
- Extract the viscera
- Harvest the giblets
- Remove and discard the intestinal tract and air sacs
- Remove and discard the trachea and crop
- Remove and discard the lungs

Potential risk factors

Chemical potential risk factors include antimicrobial treatments as well as sanitizers used to prevent cross-contamination and control microbial growth on product contact surfaces. Biological potential risk factors include pathogenic and non-pathogenic microorganisms on carcasses and equipment surfaces.

The incidence of biological potential risk factors on carcasses and equipment, and well as the change in absolute numbers, varies widely between poultry processing operations. Hargis et al. (1995) recovered *Salmonella* from 15% of ceca and 52% of crops; and 8% of crop removal devices. Byrd et al. (1998) recovered *Campylobacter* from 4% of ceca and 62% of crops. Berrang et al. (2003a) recovered 1.0 $\log_{10}$ *Campylobacter*/ml of rinse from lungs. Lillard (1990) found that the incidence of *Salmonella*-positive carcasses increased 2.4% during evisceration. Oosterom et al. (1983) found an increase of 1.5 $\log_{10}$ *Campylobacter jejuni*/g of skin and 7.0 $\log_{10}$ *Campylobacter jejuni*/g from intestinal content during evisceration. Acuff et al. (1986) found that *Campylobacter jejuni* increased 278 MPN/100 cm^3 during evisceration. Izat et al. (1988) found that evisceration increased *Campylobacter jejuni* 0.41 $\log_{10}$/1000 cm^3 on skin samples. Berrang and Dickens (2000) found a 0.3 $\log_{10}$ decrease in *Campylobacter*/ml in carcass rinses during evisceration. Berrang et al. (2003a) found that aerobic bacteria, coliforms, *E. coli*, and *Campylobacter* in carcass rinses decreased 0.5 $\log_{10}$, 0.3 $\log_{10}$, 0.67 $\log_{10}$, and 0.3 $\log_{10}$ CFU/ml during evisceration. Lillard (1990) found that evisceration decreased aerobic bacteria and Enterobacteriaceae 0.61 $\log_{10}$ and 0.18 $\log_{10}$ CFU/ml respectively. Variations in the number of microorganisms recovered from carcasses and equipment are attributable to the differences in the processing and sanitation practices.

Carcass handling during evisceration cross-contaminates product prior to opening the body cavity and after extracting the viscera. Mead et al. (1975, 1994) recovered a marker organism from the 50^{th} revolution of the transfer point, the 450^{th} carcass to pass through the vent opener, and from head removal and lung extraction machines. Byrd et al. (2002) recovered a marker organism placed in the crops prior to live hanging from 67% of carcasses at the transfer station, 78% at viscera extraction, 92% pre-crop removal, 94% post-crop removal, and 53% after the final carcass rinse. Berrang et al. (2003a) found that the lung picks up contaminated water from the scald tank that contaminates equipment and product during evisceration. Wempe et al. (1983) recovered 2.8 $\log_{10}$ *Campylobacter jejuni*/ml from recycled carcass rinse water. Thayer and Walsh (1993) found that aerobic bacteria, Enterobacteriaceae, and *E. coli* on the probe retracting viscera from chicken

increased 0.10-0.18 log10 CFU during operation. Clouser et al. (1995a) recovered *Listeria monocytogenes* from 20% of kosher carcasses sampled post-evisceration but found no link with *Listeria monocytogenes* pre-harvest and concluded that the *Listeria monocytogenes* originated from the equipment.

The relative presence or absence of enteric microorganisms on carcasses is an indicator of sanitation process control. Jimenez et al. (2003) found that, on carcasses with visible feces, a carcass rinse reduced Enterobacteriaceae. *E. coli*, and coliforms by 0.11 $\log_{10}$, 0.10 $\log_{10}$, and 0.02 $\log_{10}$ CFU/ml respectively, and on carcasses without visible feces by 0.36 $\log_{10}$, 0.23 $\log_{10}$, and 0.18 $\log_{10}$ CFU/ml respectively. Statistical significance was achieved only for the latter case ($p \leq 0.05$). However, Fluckey et al. (2003) concluded that there is no relationship between the presence or absence of enteric microorganisms and the presence or absence of *Salmonella* or *Campylobacter* ($p > 0.05$). Lillard (1990) found that a carcass rinse decreased Enterobacteriaceae by 0.24 $\log_{10}$ CFU/ml but had no effect on the incidence of *Salmonella*.

The presence or absence of visible feces is also an indicator of sanitation process control. However, there is no direct correlation between the presence or absence of visible fecal material and the presence or absence of *Salmonella* or *Campylobacter***.** Jimenez et al. (2002) found that 12% of broiler carcasses with visible fecal contamination were *Salmonella*-positive compared to 20% without visible fecal contamination (*$p > 0.05$*) and that 37% of carcasses with visible fecal contamination were *Salmonella*-positive following the carcass rinse compared to 10% without visible fecal contamination. Fletcher and Craig (1997) found that *Campylobacter* levels on reprocessed carcasses with visible fecal contamination were 0.21 $\log_{10}$ CFU higher than reprocessed carcasses without visible fecal contamination and that the incidence of *Campylobacter* and *Salmonella* on reprocessed carcasses with visible fecal contamination was 5% and 3% lower than on reprocessed carcasses without visible fecal contamination. Blankenship et al. (1975) found no significant difference in the level of aerobic bacteria, Enterobacteriacae, and presumptive *Clostridium* spp. in carcass rinses of inspected and passed, fecal-condemned, and reprocessed fecal-condemned broiler carcasses. Bilgili et al. (2002) found no correlation between the microbiological quality of broiler carcasses and the presence or absence of visible contamination.

Evisceration systems process steps also influence the incidence of carcass contamination. Russell and Walker (1997) found visible contamination on 3% of carcasses eviscerated with the Nu-Tech® system compared to 19% eviscerated with the streamlined inspection system. Jimenez et al. (2003) found feces and/or bile on 11% and 5% of carcasses post-viscera extraction. Russell and Walker (1997) found feces on 10% of carcasses post-viscera extraction and 19% post-crop removal. Crop rupture and leakage is a significant source of contamination during evisceration. Buhr and Dickens (2001, 2002) and Buhr et al. (2000) determined that crops rupture because of greater adhesion to surrounding tissues' and that fewer crops rupture when extracted toward the head compared to extracted toward the thoracic inlet ($p \leq 0.05$).

Controls

The National Chicken Council (1992) recommends proper feed and water withdrawal, maintenance and adjustment of equipment, continuous rinsing and sanitizing, enforcing employee hygiene standards, and a whole-carcass rinse with 20 ppm free available chlorine to control biological potential risk factors during evisceration. The most common methods used to mitigate biological potential risk factors are carcass rinses, off-line reprocessing, and on-line reprocessing.

Carcass Rinses

Carcass rinses are effective interventions for removing loose material from the carcass surface during evisceration (Byrd et al., 2002). Waldroup et al. (1992) recommended a 20 ppm chlorine carcass rinse post-evisceration as part of a strategy shown to decrease microbial contamination and improve food safety. Mead et al. (1975) found that a 10-20 ppm free available chlorine rinse did not eliminate a marker organism; but, 18-30 ppm free available chlorine reduced recovery of the marker organism from the 50^{th} to the 20^{th} revolution at the transfer point. Jimenez et al. (2003) found that carcass rinses reduce visible feces and bile on post-evisceration broiler carcasses by 3.4% and 2.9% respectively. Carcass rinses can also reduce biological hazards (Notermans et al., 1980). Notermans et al. (1980) found that the incidence of *Salmonella* positive carcasses decreased 36.5% when carcass rinses were incorporated into the evisceration process compared to a 20.5% increase without carcass rinses. However, carcasses rinses are not an effective intervention against attached pathogens (Kotula et al., 1967; Mead et al., 1975).

Off-line Reprocessing

Off-line reprocessing is a manual process and addresses disease conditions and contamination that cannot be removed by other means. When properly performed, off-line reprocessing eliminates visible conditions and yields carcasses microbiologically equivalent to inspected and passed carcasses (Blankenship et al., 1975); however, reductions in microorganisms are not certain. Blankenship et al. (1993) found the microbiological quality of conventionally processed and reprocessed carcasses to be equivalent for aerobic bacteria, Enterobacteriacea, and *E. col.* With respect to *Salmonella* prevalence, the overall difference between conventionally processed and reprocessed carcasses of 5.2% was not statistically significant.

On-line Reprocessing

On-line reprocessing addresses incidental fecal and/or ingesta contamination during evisceration. Acuff et al. (1986) and Izat et al. (1988) found that an on-line carcass wash reduced *Campylobacter jejuni* 344 MPN/100 cm^3 and 0.7 $\log_{10}$ CFU/1000 cm^3 respectively. On-line reprocessing is automated and relies on washing systems in combination with antimicrobial agents to achieve desired results. Water temperature, pressure, nozzle type and arrangement, flow rate, and line speed all influence the effectiveness of the washing system. Multiple washers in series are generally more

effective then a single large washer. Bashor et al. (2004) and Kemp et al. (2001b) found that a three-stage system decreased *Campylobacter* by 0.45 $\log_{10}$ CFU/ml compared to 0.31 $\log_{10}$ CFU/ml in a single stage system ($p \leq 0.05$). Online reprocessing systems installed in one plant may not perform equally well in another plant.

The addition of antimicrobial agents generally increases the effectiveness of an on-line reprocessing system. Fletcher and Craig (1997) found that 23 ppm free available chlorine reduced the incidence of *Campylobacter*-positive carcasses from 77% to 72%; and *Salmonella*-positive carcasses from 5% to 2%. Bashor et al. (2004) found that TSP and acidified sodium chlorite decreased *Campylobacter* by 1.3 $\log_{10}$ CFU/ml and 1.52 $\log_{10}$ CFU/ml respectively ($p \leq 0.05$). Yang and Slavik (1998) reduced *Salmonella* on carcasses 1.36 $\log_{10}$ CFU with 10% TSP, 1.62 $\log_{10}$ CFU with 5% cetylpyridinium chloride, 1.21 $\log_{10}$ CFU with 2% lactic acid, and 1.47 $\log_{10}$ CFU with 5% sodium bisulfate ($p \leq 0.05$). Whyte et al. (2001b) found that 10% TSP combined with 25 ppm free available chlorine decreased *Salmonella* and *Campylobacter* by 1.44 $\log_{10}$ CFU/g and 1.71 $\log_{10}$ CFU/g respectively. On-line reprocessing is not an effective against tightly attached pathogens. Reducing tightly attached microorganisms requires longer contact times then normally occurs under commercial conditions (Morrison and Fleet, 1985; Teotia and Miller, 1975).

If properly performed, on-line reprocessing of contaminated carcasses can yield better results than off-line reprocessing, and improve food safety and the microbiological quality of raw poultry (Kemp et al., 2001a). However, if process control is not maintained, results can be mixed (Fletcher and Craig, 1997) and biological potential risk factors enhanced (Blankenship et al., 1993).

Chilling

Chilling removes the natural heat from the carcass and is complete when regulatory temperature requirements are met. Immersion and air chilling are the primary chilling technologies in use in the world today. Immersion chilling is the more common method; however, both methods acceptably decrease carcass temperature and inhibit biological potential risk factors.

Potential risk factors

Chemical potential risk factors are introduced during the immersion chilling process. Tsai et al. (1987) found that lipids account for 84-98% of the organic matter in immersion chiller water and that aldehydes, which form as these lipids auto-oxidize, react with chlorine to form chlororganics, mutagenic chemicals that potentially impact the safety and wholesomeness of poultry products. Marsi (1986) found that when free available chlorine levels are ≤50 ppm, minimal free available chlorine reacts with aldehydes and forms chlororganics. However, when free available chlorine levels ≥250 ppm, chlororganic formation rises sharply.

Biological potential risk factors exist during the chilling process as pathogenic and nonpathogenic microorganisms on the carcass and in the chiller environment. *Salmonella* and *Campylobacter* are the most common pathogenic microorganisms present on carcasses and in the immersion chiller environment. Clouser et al. (1995a) recovered *Salmonella* from 60% of carcasses pre-chill, and 57% of carcasses post-chill. Wempe et al. (1983) isolated an average of 2.20 $\log_{10}$ *Campylobacter jejuni*/ml from the chiller water. Loncarevic et al. (1994) recovered *Listeria monocytogenes* from 21% of post-chill skin samples taken from pre-chill *Listeria*-negative carcasses and determined that *Listeria monocytogenes* was a biological potential risk factor when the chlorine concentration of the chiller water was ≤10 ppm free available chlorine. Clouser et al. (1995a) found a 57% incidence in *Listeria monocytogenes*-positive kosher carcasses post-chilling compared to 7% incidence with conventional slaughter methods, found no relationship between the incidences of *Listeria monocytogenes* in the flock pre- or post-chilling, and concluded that the *Listeria monocytogenes* originated from the chiller water.

Jimenez et al. (2003) found that immersion chilling reduced Enterobacteriaceae, *E. coli*, and coliforms on non-contaminated carcasses by 0.36 $\log_{10}$, 0.89 $\log_{10}$, and 0.61 $\log_{10}$ CFU/ml in carcass rinses respectively compared 1.02 $\log_{10}$, 1.16 $\log_{10}$, and 1.23 $\log_{10}$ CFU/ml in rinses from fecal contaminated carcasses. Berrang and Dickens (2000) found that immersion chilling decreased APC, coliform, and *E. coli* in carcass rinses by 0.7 $\log_{10}$, 0.3 $\log_{10}$, and 0.4 $\log_{10}$ CFU/ml respectively ($p \leq 0.05$). Lillard (1990) found that immersion chilling decreased APC and Enterobacteriaceae by 0.92 $\log_{10}$ and 0.74 $\log_{10}$ CFU/ml.

Sarlin et al. (1998) found that *Salmonella*-negative carcasses remain negative provided they are not preceded by a *Salmonella*-positive flock and that the immersion chiller is a major site for cross-contamination between *Salmonella*-negative and -positive flocks. Jimenez et al. (2003) ($p > 0.05$) found no correlation between visible ingesta on carcasses and the presence or absence of *Salmonella* during immersion chilling. Twelve percent of carcasses with visible fecal contamination were *Salmonella*-positive following immersion chilling compared to 30% without visible fecal contamination.

Air chill systems come in two basic configurations; clip-bar and vent-stream. Allen et al. (2000) determined that microbial counts on poultry carcasses are lower in air chilling systems compared to immersion chill systems. Sanchez et al. (2002) reported the incidence of *Salmonella*-positive carcasses in air chillers at 18% compared to 24% with immersion chillers; and the incidence of *Campylobacter*-positive carcasses in air chillers at 39% compared to 48% with immersion chillers ($p \leq 0.05$). Conversely, they found that coliforms and *E. coli* in whole carcass rinses were 0.25 $\log_{10}$ CFU/ml and 0.26 $\log_{10}$ CFU/ml higher with air chillers than immersion chillers. The differences are not significant with regard to the cooling efficiency, but do affect the degree of physical contact between carcasses and the potential for cross-contamination. Mead et al. (2000) found that dispersal of a marker organism was greater in a vent-stream system was greater than in a clip-bar system. Dispersal of the marker organism decreased when water sprays were turned off.

Controls

Chemical potential risk factors introduced during the chilling process through the excessive application of antimicrobial agents can be prevented, eliminated, or reduced to acceptable levels during the chilling process. Biological potential risk factors cannot be prevented or eliminated during the chilling process; however, they can be reduced to acceptable levels.

Mulder et al. (1976) found that immersion chilling decreased *Salmonella*-positive carcasses by 5%. Acuff et al. (1986) found that immersion chilling decreased *Campylobacter jejuni* 69 MPN/100 cm^3. Berrang and Dickens (2000) found that immersion chilling decreased *Campylobacter* spp. levels 0.8 $\log_{10}$ CFU/ml. Izat et al. (1988) found that immersion chilling decreased *Campylobacter jejuni* on carcasses by 0.9 $\log_{10}$ CFU/1000 cm^3. Bilgili et al. (2002) found that immersion chilling decreased *Campylobacter* by 0.86 $\log_{10}$ CFU/ml and the incidence of *Salmonella*-positive carcasses from 20.7% to 5.7%. Lillard (1990) found that on average, immersion chilling increased the incidence of *Salmonella* by 20.7%.

More reduction in biological potential risk factors can be accomplished in a properly balanced immersion chiller than at any other processing step. Conversely, an improperly balanced immersion chiller can increase biological potential risk factors. However, regardless of how well any immersion system is operated, it cannot overcome excessive biological potential risk factors entering the chilling process. The National Chicken Council (1992) recommends that processors focus on proper water temperature and water quality to control biological hazards in the immersion chiller. Water temperature should be maintained to ensure that product temperatures are in accordance with 9 CFR 381.65. l.

Maintaining proper water quality requires balancing pH, maintaining a free available chlorine concentration, and minimizing organic matter. pH influences the diffusion of hypochlorous acid (HOCl) in solution into hydrogen (H^+) and hypochlorite (OCl^-) ions. At pH <7.5 the hypochlorite ion is favored, which increases the concentration of free available chlorine. At pH >8 the hypochlorous acid moiety is favored, which decreases the concentration of free available chlorine.

Chlorine is the most common and most effective antimicrobial intervention in use in immersion systems worldwide and the effect is directly proportional to the free available chlorine concentration. Thiessen et al. (1984) could not recover *Salmonella* from chiller water when the ClO_2 residual was ≥1.3 ppm. Wabeck et al. (1969) found that 20 ppm chlorine destroyed 3.0 $\log_{10}$ *Salmonella*/ml in solution after 4 hours but not *Salmonella* on the surface of inoculated drumsticks. Villarreal et al. (1990) found that ClO_2 could eliminate recoverable *Salmonella* from carcass rinses. James et al. (1992) found that the incidence of *Salmonella*-positive carcasses increased from 48% to 72% during immersion chilling in a non-chlorinated system compared to 43% to 46% when free available chlorine at the overflow was maintained at 4-9 ppm. Yang et al. (2001) found that 10 ppm free available chlorine eliminated *Salmonella typhimurium* and *Campylobacter jejuni* from the water in 120 and 113 minutes respectively; 30 ppm produced the same result in 6 and 15 minutes; and 50 ppm in 3 and 6 minutes ($p \leq 0.05$).

Three factors determine the amount of organic matter in the immersion chiller: flow rate, flow direction, and the cleanliness of the scald water. When the chiller is more like a pond

than a river, the water is stagnant and organic matter accumulates in the water, on the paddles, and on the sides of the chiller. Thomas et al. (1979) found that when fresh water in-flow drops to <½ gallon/bird, organic matter accumulates in the chiller water. Lillard (1980) found that more organic matter in the chiller will result in less chlorine available to kill bacteria, as it will be bound to and rendered useless by the organic matter. The recommended method for performing water replacement is with a counter-current system.

Tsai et al. (1987, 1992) found that organic matter in an immersion chiller equilibrates after 5-6 hours of operation and requires 2-3 times more free available chlorine to achieve a 2 $\log_{10}$ reduction in bacteria. Lillard (1979) calculated the concentration of organic matter at equilibrium to be 91 ppm. Allen et al. (2000) found that the concentration of organic matter increases closer to the exit and is reflected in the concentration of free available chlorine at different locations within the chiller. Filtration of recycled water reduces the level of organic matter and spares free available chlorine for bactericidal activity.

Russell (2005) recommended a pH of 6.5-7.5, a water temperature <40°F, a high flow rate, and counter-current flow direction. Waldroup et al. (1992) recommended 20-50 ppm free available chlorine in the intake water in order to reduce the total microbiological load in the chiller water. The amount of chlorine added at the intake should be sufficient to achieve 1 to 5 ppm free available chlorine at the chiller overflow.

A recent study designed to examine the prevalence and number of Campylobacter on broiler chicken carcasses in commercial processing plants in the United States (Berrang et al., 2007) can provide an indicator for the effectiveness of reducing pathogen loads during all of the steps involved in poultry processing. In the study carcass samples were collected from each of 20 U.S. plants four times, roughly approximating the four seasons of 2005. At each plant on each sample day, 10 carcasses were collected at rehang (prior to evisceration), and 10 carcasses from the same flock were collected postchill. A total of 800 carcasses were collected at rehang and another 800 were collected postchill. All carcasses were subjected to a whole-carcass rinse, and the rinse diluent was cultured for Campylobacter. The overall mean number of Campylobacter detected on carcasses at rehang was 2.66 log CFU per ml of carcass rinse. In each plant, the Campylobacter numbers were significantly ($p \leq 0.001$) reduced by broiler processing; the mean concentration after chill was 0.43 log CFU/ml. Overall prevalence was also reduced by processing from a mean of ≥30 of 40 carcasses at rehang to ≥14 of 40 carcasses at postchill. Seven different on-line reprocessing techniques were applied in the test plants, and all techniques resulted in <1 log CFU/ml after chilling. Use of a chlorinated carcass wash before evisceration did not affect the postchill Campylobacter numbers. However, use of chlorine in the chill tank was related to lower numbers on postchill carcasses ($p<0.0003$). Overall, U.S. commercial poultry slaughter operations are successful in significantly lowering the prevalence and number of Campylobacter on broiler carcasses during processing.

Conclusions

1. Physical potential risk factors are quality issues that rarely exist during poultry slaughter operations and can be eliminated or reduced to acceptable levels when good commercial practices are implemented. Physical potential risk factors present a negligible risk.

2. Chemical potential risk factors are food safety and quality issues that seldom exist during poultry slaughter operations and can be prevented, eliminated, or reduced to acceptable levels through prerequisite programs. Violative chemical residues are a pre-harvest issue and the primary chemical potential risk factor. According to the 2000 National Residue Program the incidence of violative residues was 0.11% for all classes of poultry. In 2000, U. S. poultry processors slaughtered 8,546,805,234 live poultry, which means approximately 9,481,485 poultry carcasses passed through federally inspected slaughter establishments with violative chemical residues. Chemical potential risk factors present a minimal risk.

3. Biological potential risk factors are unavoidable food safety and quality issues that continually exist during poultry slaughter operations. Biological potential risk factors are present in and on all live poultry received onto official establishments and cannot be prevented or eliminated; however, they can be reduced to acceptable levels through the application of good manufacturing practices and process control. Biological potential risk factors present a significant risk.

4. The cited data for *E. coli,* Enterobacteriaceae*, Campylobacter*, *Pseudomonas, Coliform* and APC show that more microorganisms exist in and on poultry at live receiving (LR) than at any other process step in slaughter operations. The scalding (SC) and immersion chilling (IM) steps produce the greatest overall reduction by washing microorganisms from the carcass surfaces. The feather removal (FR) and evisceration (EV) steps result in an increase from the previous steps in the number of microorganisms. However, overall microorganisms are reduced from number present when the poultry are at live receiving to when the carcasses are exiting the chiller.
5. Numerical data are not available for *Salmonella*, however, *Salmonella* prevalence follows a similar distribution pattern. No <u>single</u> process step, not matter how well controlled, can prevent, eliminate, or reduce to acceptable levels, a biological potential risk factor.

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